CANCER RESEARCH

VOL. 12 APRIL 1952 No. 4

CONTENTS

SCIENTIFIC PROCEEDINGS

AMERICAN ASSOCIATION
for
CANCER RESEARCH, INC.

New York, N.Y., April 11-13
1952

THE OFFICIAL ORGAN OF THE
AMERICAN ASSOCIATION FOR CANCER RESEARCH, INC.
Published by THE UNIVERSITY OF CHICAGO PRESS

CANCER RESEARCH

This journal is sponsored by The American Association for Cancer Research, Inc.; The Anna Fuller Fund; The Jane Coffin Childs Memorial Fund for Medical Research; The Elsa U. Pardee Foundation; and The American Cancer Society.

CANCER RESEARCH is published monthly for Cancer Research, Inc., by the University of Chicago Press, 5750 Ellis Avenue, Chicago 37, Illinois. Subscriptions are by volume only and are payable in advance. The subscription price is $12.50 per volume; the price of single issues is $1.25. Orders for less than a full volume will be charged at the single-issue rate. Postage is prepaid by the publishers on all orders from the United States and its possessions. No extra charge is made for countries in the Pan American Postal Union. Postage is charged extra as follows: For Canada and Newfoundland, 50 cents per volume (total $13.00), 5 cents per issue (total $1.30); for all other countries in the Postal Union, $1.00 per volume (total $13.50), 10 cents per issue (total $1.35).

The following is an authorized agent:

For the British Empire, except North America and Australasia:

Cambridge University Press, Bentley House, 200 Euston Road, London, N.W. 1, England. Prices of subscriptions and of single copies may be had on application.

Business communications, remittances (in United States currency or its equivalent), and subscriptions should be addressed to THE UNIVERSITY OF CHICAGO PRESS, 5750 Ellis Avenue, Chicago 37, Illinois. All other communications should be addressed to Harold P. Rusch, M.D., McArdle Memorial Laboratory, University of Wisconsin, Madison 6, Wisconsin.

Claims for missing numbers should be made within the month following the regular month of publication. The publishers expect to supply missing numbers free only when losses have been sustained in transit and when the reserve stock will permit.

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Entered as second-class matter, February 15, 1949, at the post office at Chicago, Ill., under the Act of March 3, 1879.

Acceptance for mailing at special rate of postage provided for in United States Postal Act of October 3, 1917, Section 1103, amended February 28, 1925, authorized June 1, 1950.

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GROWTH OF MOUSE SARCOMA 180 IN THE ADRENALECTOMIZED ADULT HAMSTER (*MESOCRICETUS AURATUS*).

FREDERIC J. AGATE, JR.,* and FAY E. AGATE* (introduced by Samuel Graff). (Departments of Anatomy and Biochemistry, Institute of Cancer Research, and Francis Delafield Hospital, Columbia University, New York, N.Y.)

Successful transplantation of mouse Sarcoma 180 into hamsters has been reported. However, the percentage of transplants which continued growth decreased greatly with the age of the animals, and subcutaneous transplants did not grow in adult hamsters.

Four groups of adult hamsters of both sexes were adrenalectomized, implanted with a 10-mg. pellet of desoxycorticosterone acetate, and placed on a 0.9 per cent sodium chloride solution substituted for drinking water. The day following operation each animal was implanted subcutaneously with a fragment of Sarcoma 180 weighing 20–30 mg. from Paris strain mice. At the same time an equal number of intact animals was implanted with sarcoma, half of the controls having been subjected to a sham operation and implanted with a pellet of desoxycorticosterone acetate.

In three groups which were implanted in the winter, early spring, and late fall, respectively, the tumor continued to grow in about 90 per cent of the adrenalectomized hamsters. Between the 9th and 30th day after implantation the animals became comatose and were sacrificed. Adrenalectomized animals which were not sarcoma-implanted, bearing a desoxycorticosterone pellet and maintained on 0.9 per cent sodium chloride, remained in good health throughout the experiment.

In about 10 per cent of the sarcoma-injected controls, the tumor continued to grow.

In one group implanted during hot weather in the summer the sarcoma grew in only 50 per cent of the adrenalectomized hamsters and in about 20 per cent of the controls.

THE EFFECT OF INHERITED FACTORS, THE MILK AGENT, AND PHYSIOLOGICAL STATE ON PHOSPHORUS METABOLISM OF ENDOCRINE GLANDS OF MICE.

S. ALBERT and R. M. JOHNSON.* (Richard Cohn Radiobiology Laboratory, Detroit Institute of Cancer Research, Detroit 1, Mich.)

Inherited susceptibility, hormonal influences, and the milk agent are concerned with the development of spontaneous mammary carcinoma in mice. Information on the mechanisms of action of these factors is inadequate. Preliminary observations indicate that inherited factors, the milk agent, and physiological state play a role in the phosphorus metabolism of the endocrine glands of mice.

Radioactive phosphorus was administered to virgin and breeder mice of the C57, DBA— (DBA minus the milk agent), and DBA strains, and to pregnant DBA mice, and the mice were sacrificed 1, 4, 17, or 48 hours later. Total phosphorus and P^{32} content of the ovaries, adrenals, thyroids, and pituitary glands were determined.

DBA− mouse ovaries and adrenals contained amounts of phosphorus similar to that found in corresponding organs of DBA mice. This amount was lower in the ovaries and higher in the adrenals than in corresponding glands of C57 mice. The phosphorus content of thyroids of DBA− and DBA virgins differed but was lower in each than in these glands in C57 mice. It may be concluded that inherited factors influence the phosphorus content of ovaries, adrenals, and thyroids in the strains studied.

In these strains the presence of the milk agent was associated with a decrease in phosphorus content of the thyroids, with an increased P^{32} uptake in virgin animal thyroids and with a decreased P^{32} uptake in the ovaries and adrenals of breeders.

Pregnancy was accompanied by a large increase in the rate of P^{32} uptake in all the endocrine glands examined.

THE ROLE OF HYPOTENSION IN THE ACTION OF A BACTERIAL TOXIN ON TUMORS.

Glenn H. Algire, Frances Y. Legallais,* and Belle F. Anderson.* (National Cancer Institute, Bethesda, Md.)

The possible role of hypotension in tumor damage induced by a bacterial polysaccharide was investigated by use of the transparent chamber technic and an indirect method for measurement of peripheral blood pressure. *In vivo* microscopic observations on blood flow in sarcomas and surrounding normal tissues were made prior to and following intraperitoneal injection of bacterial polysaccharide preparations at dosages ranging from 8 to 270 μg. Measurements of changes in the percentage of functional tumor capillaries were correlated with determinations of systolic blood pressure in arteries of normal tissue adjacent to tumors growing within the transparent chambers.

The results showed that the degree and duration of hypotension were directly correlated with the dosage. Hypotension was accompanied by tumor ischemia which persisted during the period of low blood pressure. Evidence of irreversible ischemic damage to tumor capillaries, as indicated by stasis, hemorrhage, or thrombus formation, occurred after about 2 hours or more of hypotension. The extent of tumor damage was proportional to the degree and duration of hypotension. Repeated injections at intervals of 1–8 days produced a resistance which was associated with decreased degree and duration of hypotension.

It is concluded that the tumor-necrotizing effect of this agent is brought about by the ischemia and circulatory stasis induced by hypotension.

THE EFFECT OF PANTOTHENATE INTAKE ON CONJUGATION AND TOXICITY OF CARCINOGENIC AMINES.

J. B. Allison, A. W. Wase,* and J. F. Migliarese.* (Bureau of Biological Research, Rutgers University, New Brunswick, N.J.)

The effects of varying the intake of pantothenate on conjugation and toxicity of the carcinogens, β-naphthylamine and 2-aminofluorene, are being studied in the rat and in the dog. Excretion of the conjugated form of β-naphthylamine was increased in the dog from 5 to 16 to 33 per cent as the dietary pantothenate varied from 0 to 0.2 to 20 mg/day/kg of body weight. Given a constant intake of pantothenate, the per cent conjugation decreased with increasing dosage of the carcinogen. The per cent conjugation of β-napthylamine was higher in the rat than in the dog. In the rat, raising the pantothenate intake did not increase the per cent conjugation markedly, but did increase the total excretion of β-naphthylamine and the conjugated form. This carcinogen was much more toxic to rats fed a pantothenic acid-deficient diet than to those saturated with the vitamin. Similarly, saturating dogs with pantothenate had a protective effect. Dogs receiving 20 mg. pantothenate/day/kg body weight and 400 mg. of β-napthylamine/day for 6 months showed no pathology. Animals on lower pantothenate intakes showed evidence of damage to the bladder and liver. Previous studies had demonstrated that increasing the riboflavin content of the diet raised the excretion of conjugated 2-aminofluorene and of nonvolatile phenols in dogs and in rats. Recent data have demonstrated that maximum excretion of conjugated 2-aminofluorene is also dependent upon dietary pantothenate.

β-GLUCURONIDASE STUDIES IN BLAST-CELL LEUKEMIAS.

A. J. Anlyan and B. Sherman.* (Chemotherapy Service, Memorial Cancer Center, and the Division of Experimental Chemotherapy, Sloan-Kettering Institute, New York, N.Y.)

Previous work has demonstrated differences in β-glucuronidase activity of the white blood cells in human subjects. Normal values have been established in a control series, and consistent differences shown in granulocytic and lymphocytic leukemias of adults.

The present study consists of an attempt to differentiate the blast-cell leukemias of children at a stage of the disease when the microscopic picture will not specify whether the malignant cells are of the lymphocytic or granulocytic series.

The buffy coat β-glucuronidase activity in one group of these patients was within or below the normal range, and in another group was abnormally high. These cases are being followed at intervals during therapy with the various agents used at the Memorial Hospital—A-methopterin, ACTH, and cortisone, in the hopes of finding correlations between β-glucuronidase levels and response to therapy.

Thus far, in two patients there appeared to be a correlation between the β-glucuronidase activity per gram of buffy coat and the clinical state of the disease, in that the abnormally high levels which were found at time of relapse fell when the patient went into remission.

INFLUENCE OF SARCOMA 180 ON CARCINOMA 755 IN THE MOUSE. William Antopol, Samuel Graff, Gerard Zagal,* and Fay Agate.* (Laboratories of the Joseph and Helen Yeamans Levy Foundation, Beth Israel Hospital, and the Department of Biological Chemistry, Francis Delafield Memorial Hospital, Institute of Cancer Research, College of Physicians and Surgeons, Columbia University, New York, N.Y.)

Tumors, whether spontaneous or experimentally transplanted, bear a parasitic relationship to the host. This relationship is more complex than tumor-host competition for essential metabolites. The experiments described are concerned with the influence on each other of two tumors, Sarcoma 180 and Carcinoma 755, growing simultaneously in C57 mice. It was found that Sarcoma 180 inhibited Carcinoma 755, while Carcinoma 755 enhanced the growth of Sarcoma 180. 8-Azaguanine, which inhibits the growth of Carcinoma 755, did not influence Sarcoma 180 growing in the same mouse, nor did azaguanine prevent Carcinoma 755 stimulation of Sarcoma 180 growth. The guanase present in Sarcoma 180 in high concentration deaminates 8-azaguanine and might account for the specificity of the drug. Apparently, the guanase of Sarcoma 180 does not neutralize the effect of 8-azaguanine reaction on Carcinoma 755, since there is no diminished response of Carcinoma 755 to 8-azaguanine when this tumor and Sarcoma 180 are grown in the same mouse; in fact, there appears to be an exaggeration of the effect of 8-azaguanine on Carcinoma 755. The Sarcoma 180-stimulating substance produced by the carcinoma does not seem to involve the mechanism susceptible to 8-azaguanine. The possibility remains that there is a competition between the two tumors for a unique unidentified nutritional element necessary for Carcinoma 755 growth. The findings indicate further biologic differences between tumors and illustrate humoral or systemic effects of neoplasia.

PROGRESSIVE GROWTH STAGES IN THE DEVELOPMENT OF SPONTANEOUS THYROID TUMORS IN THE SWORDTAIL *XIPHOPHORUS MONTEZUMAE*. Olga Aronowitz,* Martha Edgar,* and Myron Gordon. (Genetics Laboratory of the New York Aquarium, New York Zoölogical Society, New York, N.Y.)

The development of the thyroid tumor has been studied in swordtails, *Xiphophorus montezumae*. Fishes were sacrificed monthly from day of birth to 11 months of age. The progressive development of the tumor is as follows:

(*a*) Day-old fish have 20–30 thyroid follicles, lined by squamous or low cuboidal epithelium, scattered in the stroma around the ventral aorta, but not in the gills. (*b*) The follicle cells increase in size and become high columnar. (*c*) The number of thyroid follicles increases; the blood capillaries in the region of the follicles become and remain engorged with red blood cells. (*d*) The epithelial cells of the new follicles simultaneously increase in number and size; some of the blood capillaries rupture, and individual red blood cells are found close to the follicular cells. (*e*) The follicle configuration becomes distorted. (*f*) The follicles disintegrate. (*g*) Tumorous growths develop, composed primarily of afollicular epithelial cells, microfollicles, and hemorrhages. (*h*) Muscles in the region of the ventral aorta are invaded by tumorous afollicular epithelial cells. (*i*) Tumor cells invade the deeper musculature, the gill filaments, cartilage, and bone. (At this stage the thyroid tumor is visible externally.) (*j*) Death may be due to destruction of visceral gill arches and the consequential interference of normal respiration.

The known differences in the thyroidal tissue in wild swordtails fixed at the site of capture, the Río Axtla, Mexico, their F_1 laboratory-reared offspring, and their seventh generation descendants will be discussed.

THE EFFECT OF FLAVONOIDS ON THE RADIOSENSITIVITY OF MALIGNANT TUMORS. I. Arons, A. Oppenheim, J. Freeman,* R. Williams,* B. Sokoloff, and W. H. Eddy. (Harlem City Hospital, N.Y. and Southern Bio-Research Laboratory, Florida Southern College, Lakeland, Fla.)

Contact radiation, a single dose of 10,000 r and 15,000 r, was applied to rat Sarcoma 39 and

Crocker rat carcinoma, average size tumor 18–19 c.mm. Radiation factors were: 60 kv., 0.12 mm. cu. filter, 3 cm. distance from the target to the center of the tumor. The field was 2 cm. in diameter with 450 r/min. Seventy-eight rats served as control, 78 rats were given citrus flavonoid compound, 10 mg/100 gm/weight, for 7 days prior to the exposure and for 21 days after radiation. All tumors of the control and treated groups of Sarcoma 39 were destroyed completely by radiation by both of these doses. Crocker rat carcinoma was more radioresistant. Contact radiation of 10,000 r destroyed completely 40 per cent of tumors in the control and 53 per cent in the treated group. Contact radiation of 15,000 r destroyed all tumors in both groups with the mortality of 77.7 per cent in the control and 22.2 per cent in the group receiving flavonoids. Flavonoid therapy does not decrease the radiosensitivity of Crocker carcinoma and Sarcoma 39 but markedly increases the tolerance of rats to contact radiation. The analysis of the case histories submitted to deep radiation therapy and receiving flavonoids indicates that there is no decrease in the radiosensitivity of malignant growth but a definite increase in tolerance.

TUMORS OF THE EPIDIDYMIS AND OF THE UTERUS IN HAMSTERS TREATED WITH DIETHYLSTILBESTROL AND TESTOSTERONE PROPIONATE. R. L. Bacon (introduced by A. D. Dulaney). (Division of Anatomy, University of Tennessee, Memphis, Tenn.)

In connection with an investigation of the endocrine factors involved in the production and control of renal tumors in hamsters, ten females and eleven males received diethylstilbestrol and testosterone propionate simultaneously from pellets implanted subcutaneously on the 50th day of life. The animals died or were sacrificed after from 223 to 543 days of treatment. Cystic glandular hyperplasia was found in the uteri of all females and extensive hyperplasia of muscle, connective tissue, and epithelium throughout the reproductive tract of the males. In addition, multiple leiomyomas were found in one or both uterine horns of six of the ten females and leiomyomas of one or both epididymides in five of the eleven males. The tumors from the two sexes were histologically indistinguishable. Among twenty females and 60 males treated for equal or greater lengths of time with diethylstilbestrol alone, a single tumor nodule developed in the uterus of each of two females and none in any of the males. No tumors of the uterus or of the epididymis have been found in any untreated animals in our colony.

These tumors appear to be of the same type as those which, in the guinea pig, may be produced by treatment with estrogen and prevented by simultaneous administration of testosterone.

STUDIES ON THE UPTAKE OF P^{32} BY MOUSE MAMMARY CARCINOMA. Cyrus P. Barnum, Robert A. Huseby, and Halvor Vermund.* (Departments of Physiological Chemistry and Pathology, University of Minnesota Medical School, Minneapolis, Minn.)

AZF_1 mice bearing a transplanted mammary carcinoma were injected intraperitoneally with a solution containing P^{32} and were then sacrificed at intervals varying from 15 minutes to 16 hours later. The healthy tumor tissue was removed, homogenized, and fractionated into a nuclear fraction and three cytoplasmic fractions. The nucleic acids, phospholipids, and "phosphoproteins" of each of these fractions were isolated and their specific activity compared to that of the inorganic phosphorus of the tumor tissue. As was previously observed for mouse liver, the pentosenucleic acid (PNA) of the nuclei shows a much more rapid increase in specific activity than any of the other nucleic acid fractions. Of all the organic fractions studied, the "phosphoprotein" fraction shows the most rapid increase in specific activity and at 15 minutes is about 5 times that of the nuclear PNA. The uptake of P^{32} by the phospholipids is much less than was observed in liver tissue; however, there are differences between the phospholipids of the cytoplasmic fractions that were not apparent in the liver studies. By the end of 16 hours, with this particular tumor line, all the fractions studied have very nearly reached equilibrium.

TUMOR GROWTH IN MICE WITH AND WITHOUT THE MAMMARY TUMOR AGENT. Morris K. Barrett and Margaret K. Deringer.* (National Cancer Institute, Bethesda, Md.)

A mammary tumor of strain C3H origin has been transplanted into over 3,000 hybrids derived from that strain. Suitable tests showed that the tumor contained the agent during the course of this work. The hosts were reciprocal F_1 hybrids between strain C3H, with the agent, and strain C, without the agent, and backcross mice obtained by mating such F_1 hybrid females with strain C males. No test was made for the presence of the

agent in these hosts, but, in accordance with well established principles, it was assumed that those F_1 hybrids having a strain C3H mother carried the agent and so also did the backcross mice derived from such F_1 hybrids. Conversely, the agent would be absent from those animals having a strain C mother or maternal grandmother.

The tumor grew in all F_1 hybrids, but when the agent was present in the host the tumors grew more rapidly and the hosts died earlier than was the case when the agent was absent. Appropriate experiments indicated that such differences could be produced or nullified by foster nursing.

The tumor grew in a small percentage of backcross hosts; when the agent was present in the host the percentage was higher and the animals survived a shorter time than when the agent was absent from the host.

Other interpretations are not excluded, but it seems that the most likely explanation of these observations depends upon the presence or absence of the agent in the host.

METABOLISM OF GLYCINE IN MICE MAINTAINED ON CHRONIC CALORIC RESTRICTION. A. D. Barton* and H. P. Rusch. (McArdle Memorial Laboratory, University of Wisconsin, Madison 6, Wis.)

The metabolism of glycine in normal mice and in mice maintained on chronic caloric restriction has been studied, after a single injection of glycine-2-C^{14}, by following the specific activity of the uncombined glycine in liver, muscle, and plasma, as well as the specific activity of the expired carbon dioxide.

By the use of methods described previously (Proc. Soc. Exper. Biol. & Med., 77: 481, 1951), it was found that in the mice maintained on chronic caloric restriction the size of the glycine "pool" was 47 mg/100 gm of body weight, of which 15 mg/100 gm was uncombined glycine; the turnover rate was 26 mg/100 gm/hour, and the rate of oxidation to yield respiratory carbon dioxide was 6 mg/100 gm/hour. In the normal mice, the size of the glycine pool was 31 mg/100 gm, of which 10 mg/100 gm was uncombined glycine; the turnover rate was 22 mg/100 gm/hour, and the rate of oxidation was 3 mg/100 gm/hour.

The weight of the restricted mouse was approximately two-thirds of that of the normal mouse, so that, when calculated per mouse, the size of the glycine pool in the restricted mouse was similar to that in the normal mouse, despite the restriction in food intake. However, even when calculated on this basis, the oxidation of glycine to yield respiratory carbon dioxide was approximately 50 per cent greater in the restricted mouse.

PARENTERAL AUREOMYCIN THERAPY IN CANCER. Jeanne C. Bateman, Calvin T. Klopp, and J. Roland Barbario.* (George Washington University School of Medicine, Washington, D.C.)

Aureomycin has been reported to alleviate diarrhea and prolong life in radiated mice and dogs. These findings suggested its use as an adjuvant agent in treatment of cancer patients receiving x-ray and massive HN2 therapy. Because of reports by Sokoloff that large doses of aureomycin suppressed transplanted rat tumors, aureomycin was injected directly into the artery leading to the tumor site through an indwelling polyethylene cannula. If this was not practical, intravenous administration was employed. Thirty-four courses of aureomycin were administered to 31 patients with far advanced cancer: combined with HN2, 13 times; with x-radiation, 8 times; and given alone, 13 times. Total doses ranged from 4.5 to 64 gm. given in 4 to 61 days.

Modification of treated cancer was of such degree that surgery could be performed on five patients who had been declared inoperable. Five patients died during or shortly following therapy. Administration of aureomycin was followed by some gross and nonspecific microscopic effects on tumors and, in some cases, appeared to potentiate effect of HN2 or x-ray on cancer.

Jaundice resulted from administration of aureomycin. Its development was related to duration of therapy. Results of liver function studies will be presented. Findings were compatible with intrahepatic obstructive type of jaundice. There was an associated rise in NPN and no bilirubinuria. Jaundice was quickly reversible with reduction of aureomycin except when severe impairment of renal function due to other causes was present. It was postulated that aureomycin competes with bile for excretion by the liver and kidney cells.

FURTHER STUDIES ON THE ROLE OF THYROXINE IN CHEMICAL CARCINOGENESIS. Roy Bather* and W. R. Franks. (Banting and Best Department of Medical Research, University of Toronto, Toronto, Canada.)

Following previous observations that the incidence of tumors at the site of injection of chemical carcinogens is reduced by a single injection of thyroxine or other metamorphosing hormones given with the carcinogen, metabolic studies have shown

that the rate of disappearance of carcinogen from mice is significantly increased by the thyroxine treatment. Four weeks after the injection of 1,2,5,6,-dibenzanthracene into mice treated or untreated with the thyroxine, the amount of carcinogen remaining in the carcasses, estimated spectrographically, is significantly lower in the thyroxine-treated animals. Conversely, dibenzanthracene exerts an inhibitory effect on the toxic action of thyroxine, with anoxia survival time as the criterion.

When the dose-response curve of mice to the sarcomagenic action of dibenzanthracene is studied (injections of 5, 16, 48, and 1,000 μg. DBA), it is found that simultaneous administration of thyroxine significantly lowers the tumor response. This is also true if thyroxine is given at a different site in the animals. Thiouracil feeding, on the other hand, markedly increases the incidence of dibenzanthracene tumors produced by a dose of 1 mg. of DBA.

Since thyroxine has been found to have little or no effect on the growth of already established tumors (transplanted sarcomas), it would appear that it may play an important part in the normal cell's defense against chemical sarcomagenesis by increasing the metabolic destruction of the carcinogen at least when the latter is injected subcutaneously in submaximal doses.

INHIBITION OF GONADOTROPHINS DURING TUMOR GROWTH IN THE RAT.

R. W. Begg and A. G. Stewart.* (Department of Medical Research, University of Western Ontario, London, Canada.)

The testicles, prostate, and seminal vesicles of male tumor-bearing rats are smaller than those of the controls but respond to injected pregnant mare serum (PMS). The presence of a tumor inhibits the maturation of ovary and uterus in the young female rat, and PMS overcomes the inhibition. It is suggested that the presence of a tumor produces a deficiency of gonadotrophic hormones in the rat. The deficiency also is demonstrable when the control rats are pair-fed to the tumor rats and when the tumor-bearers are force-fed to maintain carcass weight.

SOME FACTORS GOVERNING THE ALBUMIN CONTENT IN HUMAN PLASMA.

Peter Bernfeld* and Freddy Homburger. (Cancer Research and Cancer Control Unit, Tufts College Medical School, Boston, Mass.)

The plasma albumin concentration in a patient population of three different hospitals was studied by means of electrophoretic analyses. The results obtained in the first 70 individuals of this group show that patients with cancer (26 cases) have a lower average plasma albumin content than individuals without cancer. The following observations, however, demonstrate that this phenomenon may also be related to factors other than disease. The albumin content of the plasma of patients without cancer who were hospitalized in a Jewish hospital is significantly lower than that of noncancer patients from other hospitals. This may be due to the nature of the diet (kosher or nonkosher), appetite, and psychological factors. Furthermore, the comparison of the ambulatory with the nonambulatory patients without cancer shows a lower average albumin content in the plasma of the bed patient. On the other hand, the albumin distribution is the same in patients under or over 40 years of age, and no differences are found when the effect of sex is analyzed.

It appears from these observations that the plasma albumin content is markedly influenced by factors such as nutrition, the extent of physical activity of the patient (e.g., ambulatory or nonambulatory state), and perhaps others. The plasma albumin content cannot, therefore, be considered specific for any disease state, unless these factors have been adequately controlled.

THE HEMATOLOGICAL RESPONSE OF LEUKEMIC AND NONLEUKEMIC PATIENTS TO INTRAVENOUS HISTAMINE.

H. R. Bierman, K. H. Kelly,* F. L. Cordes,* L. P. White, and A. Littman.* (Laboratory of Experimental Oncology, National Cancer Institute, and the Division of Medicine, University of California School of Medicine, San Francisco, Calif.)

The intravenous administration of 0.1–0.3 mg. of histamine over 30 seconds in nonleukemic patients is followed by a marked arterial leukopenia within 30 seconds which persists for 1–3 minutes. The leukopenia does not appear in the venous blood sampled from the pulmonary conus or a peripheral vein until 30–60 seconds after the arterial count falls.

The intravenous administration of histamine in identical fashion to seventeen consecutive patients with lymphatic leukemia failed to produce any significant change in leukocyte count in either the venous or arterial blood. Two of three patients with myelogenous leukemia exhibited a slight leukopenia which appeared 1–2 minutes after the histamine and slowly returned to the original levels. Three patients with monocytic leukemia responded as the nonleukemic patients.

One patient with lymphatic leukemia received 0.9 mg. histamine, intravenously, and despite the extreme reaction the *arterial* leukocyte number remained relatively constant. Large fluctuations in leukocyte number related to the respiratory effect were observed in the *venous* blood samples. The data suggest that the lung leukocyte removal mechanism does not respond to histamine in patients with lymphatic leukemia.

PURINE ANTAGONISM AND DIFFERENTIAL TOXICITY OF SOME 2-AZAPURINES IN MOUSE TUMOR TISSUE CULTURES. JOHN J. BIESELE, RUTH E. BERGER,* and MARILYN CLARKE.* (Cell Growth Section, Division of Experimental Chemotherapy, Sloan-Kettering Institute for Cancer Research, New York 21, N.Y.)

Marked differential damage to mouse Sarcoma 180 cells in tissue culture was caused by 2-azaadenine. A far lower and less selective toxicity was displayed by 2-azahypoxanthine and 8-oxy-2-azaadenine. The toxic effects of 2-azaadenine on Sarcoma 180 cells could be blocked with adenine sulfate. The antagonism was noncompetitive. The toxic effects of 2-azaadenine were not blocked with hypoxanthine, 2-azahypoxanthine, or 8-oxy-2-azaadenine at the concentrations tested. No significant increase in frequency of mitotic or chromosomal abnormalities was seen in mouse Sarcoma 180 or embryonic skin tissue cultures treated with 2-azaadenine. These results reinforce those previously obtained with 2,6-diaminopurine and 2-chloroadenine in emphasizing the significance in Sarcoma 180 metabolism of position 2 in adenine.

PEPTIDASE ACTIVITY IN THYMUS GLANDS OF NORMAL AND LEUKEMIC MICE DURING GROWTH AND AGING. MARION K. BIRMINGHAM,* BERNARD GRAD, MARY E. PUTNAM,* and KARL STERN.* (Gerontologic Unit, Allan Memorial Institute of Psychiatry, McGill University, Montreal, Canada.)

The peptidase activity of thymus homogenates was determined in 63 normal (Rockland all-purpose–RAP) mice from 12 to 427 days of age and in 49 Ak mice from 18 to 376 days of age. All mice were apparently healthy, except 12 animals of the Ak strain, which were acutely ill with spontaneous lymphatic leukemia. The peptidase activity was determined by the method of Grassmann and Heyde (Ztschr. Physiol. Chem., **183**: 32, 1929), with 0.05 M glycylglycylglycine as substrate.

In RAP mice between 1 and 14 months of age, the peptidase activity per milligram of thymus decreased significantly with increasing age; this decrease did not occur in Ak mice. The peptidase activity was higher in Ak thymus than in the glands from the RAP strain in both young and old animals. This difference was particularly marked at the age period when the development of lymphatic leukemia is most frequent in Ak mice. However, there was no significant difference between the thymic peptidase activity of mice with active leukemia (as determined by greatly enlarged thymus, lymph nodes, or spleen) and that of apparently normal Ak mice.

TRANSFER OF THE AGENT FOR MAMMARY CANCER IN MICE BY THE MALE. JOHN J. BITTNER. (Division of Cancer Biology, Department of Physiology, University of Minnesota Medical School, Minneapolis 14, Minn.)

Females of inbred strains without the agent for mammary cancer differ in their sensitivity to infection by males with the agent, and males of different cancerous strains vary in their ability to infect females of either the same or other stocks.

Sixteen of 27 C females developed mammary cancer when mated with Z (C3H) males and five of fifteen when A males were used; 42 Zb females were crossed with either Z or D males, and one had cancer. Z or A males were mated to 49 Ax and 62 B females, and none either became infected or died cancerous. Of 493 F_1 and ZBC hybrids (Ax $\times$ Zb stocks), seven had tumors when bred with Z males. In the groups where cancerous mice were observed, others became infected but died noncancerous.

As soon as the females become "infected" from the male, the agent is transferred in their milk to their progeny; comparable to the injection of an extract with the agent into adult mice. Infection may not occur before the eighth litter, and the time of development of mammary cancer may be correlated with the number of litters born within a given period. The agent may be recovered from the tumors from either the infected mothers or their progeny.

Should there be a "nursing factor" for breast cancer in humans, and it behaved like the agent for mammary cancer in mice (transferred in the milk, blood, at coitus, etc.), it would be most difficult to demonstrate in the human population.

DEHYDROGENASE ACTIVITY OF LIVER AND KIDNEY SLICES IN TUMOR MICE. MAURICE M. BLACK and FRANCIS D.

SPEER.* (Department of Pathology, New York Medical College, New York, N.Y.)

The *in vitro* dehydrogenase activity of liver and kidney slices from control and tumor-bearing mice was determined by their reduction of tetrazolium chloride (TTC). The mice studied included C3H and CFW animals with and without spontaneous mammary carcinomas, as well as mice of the DBA strain with and without a transplantable mammary carcinoma. In addition, the ratio of the kidney/liver dehydrogenase activity was also determined.

It was found that the reduction of TTC by the liver slices from tumor-bearing mice tended to be slightly higher than that found in the control series. In contrast, the kidney slices yielded somewhat lower values in the tumor, as opposed to those in the control group. Much more striking, however, was the difference in the ratios of kidney/liver in the two series. A decided tendency toward lower ratios was found in the tumor series. This difference was as marked in animals bearing small tumors as in those with larger tumors.

THE HISTOPATHOLOGIC EFFECTS OF ACTH OR CORTISONE IN PATIENTS WITH LYMPHOMAS. MATTHEW BLOCK* and LEON O. JACOBSON. (Department of Medicine, University of Chicago, Chicago 37, Ill.)

Twenty-one cases of lymphomas, primarily acute leukemia, have been treated with ACTH or cortisone. About two-thirds were benefited—the highest percentage of remissions occurring in acute leukemia. The patients had biopsies of the marrow and in some cases of liver, spleen, and lymph nodes. In each case a control biopsy was obtained immediately prior to treatment and further biopsies as frequently as possible thereafter.

In general the most marked histological changes were seen in the patients who developed a clinical remission. These changes were particularly prominent in patients with acute leukemia and with the acute phase of chronic leukemia. The tissues of the patients prior to treatment consisted of so dense a structureless mass of leukemic tissue that the organ was not easily recognizable. During remission, fat appeared in the marrow. Erythroblasts, granulocytes and their precursors, and megakaryocytes were found in approximately normal numbers and degrees of maturation. However, small islands of stem cells were almost invariably demonstrable at the same time.

Similarly, a normal architecture, including recognizable white pulp and cords and sinuses in the red pulp, were demonstrable in the spleens of patients undergoing a remission. Islands of stem cells were always seen in the white pulp, in even the most complete remission.

Usually the tissues of patients not developing a remission, even if they had been treated successively previously, did not show any change following treatment.

DEVELOPMENT OF ACUTE LEUKEMIA IN HUMAN ADULTS. MATTHEW BLOCK* and LEON O. JACOBSON. (Department of Medicine, University of Chicago, Chicago 37, Ill.)

Observations on the development and histogenesis of acute leukemia in the human adult are extremely rare. In the last 2 years nine adults have been studied by means of serial blood counts and serial biopsies of the marrow and in some cases of liver and spleen during periods of up to 18 months before the acute leukemia became manifest.

Seven were menopausal females, four of whom had multiple allergies varying from asthma to drug idiosyncrasy. All had thrombopenia and neutropenia, which in some had been noted as long as a year preceding development of leukemia. Each patient was anemic for prolonged periods, and this anemia usually was not benefited by transfusions, indicating the possibility that abnormal hemolysis antedated the leukemic state.

Hypercellularity and maturation arrest in the granulocyte series were seen months prior to leukemic metaplasia of the marrow. In approximately half the patients the marrow was solidly cellular during the preleukemic phase. One patient had an atrophic and then an aplastic marrow for several months, which became solidly leukemic in a period of only 6 days. Liver and spleen did not undergo leukemic transformation as early as did the marrow and in some patients at autopsy was less involved than the marrow.

EFFECT OF ACTH UPON INDUCED LEUKEMIA IN DBA MICE. MATTHEW BLOCK and GEORGE TAKANO* (introduced by Leon O. Jacobson). (Department of Medicine, University of Chicago, Chicago 37, Ill.)

After leukemia was induced in mice by painting with methylcholanthrene, the animals were divided into three groups. Group I (24 mice) was injected with 0.1 mg. of ACTH in saline 3 times daily; group II (fifteen mice) was similarly injected with saline; and group III (ten mice) was untreated. All survivors were sacrificed at 21 days.

There was no significant difference in white and differential counts among the three groups. Sur-

vival time was not significantly altered by treatment. ACTH-treated animals had a much higher incidence of infection, undoubtedly contributing to the mortality rate of this group. Nevertheless, these animals seemed better nourished and more active. The tissues of all animals dying during the first 14 days were similar.

In group I, one of three animals at 14–17 days, one of two at 18–20 days, and five of eight at 21 days showed histologic evidence of regression of their leukemia and the development of myeloid metaplasia in liver and spleen. In group II, one of three animals at 14–17 days and one of five at 21 days showed some questionable evidence of a similar change. One of ten animals in group III had a regression of leukemia.

THE EFFECT OF FEEDING HABITS ON CARCINOGENESIS. R. K. Boutwell and H. P. Rusch. (McArdle Memorial Laboratory, University of Wisconsin, Madison, Wis.)

A study was made of the importance to carcinogenesis of the characteristic feeding patterns accompanying caloric restriction. The normal feeding patterns of the mice are as follows: mice fed at a high calorie level have food available to them throughout the day in contrast to mice restricted in calories; at levels of restriction of about 60 per cent of the ad libitum caloric intake, mice will consume a 24-hour allotment of diet within ½ hour. Therefore, tumor induction was studied under two conditions. In one case, mice were trained to confine their food consumption to a 1½-hour period from 9:00 to 10:30 A.M. and a similar 1½-hour period at night. After a short period of adaptation, these mice consumed as much diet as the control group fed ad libitum. In the second approach, the time required for consumption of a low calorie diet was extended to 24 hours by dilution with powdered cellulose. As much as 6 parts by weight of cellulose was added to 4 parts of the diet. Tumors were induced by painting a 0.3 per cent solution of benzpyrene in benzene on the backs of mice twice weekly for 35 applications. There were 48 mice per group, and the experiment was repeated. Hastening the ingestion of the high caloric diet or prolonging the ingestion of the restricted diet by dilution had no consistent effect on the formation of tumors.

DIAMINO DICHLOROPHENYL PYRIMIDINES IN MOUSE LEUKEMIA. Joseph H. Burchenal, Sarah K. Goetchius,* C. Chester Stock, and George H. Hitchings. (Division of Experimental Chemotherapy, Sloan-Kettering Institute, New York, N.Y., and The Wellcome Research Laboratories, Tuckahoe, N.Y.)

The antitumor activity against Sarcoma 180 in mice of certain diamino phenyl pyrimidines synthesized as anti-malarials was detected by Clarke *et al.* For this reason a series of these compounds was studied for effects on transplanted mouse leukemia. Certain of these derivatives were effective in prolonging the survival time of mice inoculated with the Ak_4 strain of leukemia and caused a rapid fall in the number of leukemic leukocytes in the peripheral blood when administered in the late stage of the disease. For maximum effect, the 5-phenyl substituent should be halogenated in the 3′ and 4′ positions. Compounds with a methyl or ethyl group in the 6-position of the pyrimidine ring were most effective. In mice with advanced leukemia of the Ak_4 (A-methopterin-sensitive) or the Ak4R (A-methopterin-resistant) strains, the administration of a single dose of 10 mg/kg of 2,4-diamino-5-(3′,4′-dichlorophenyl)-6-methylpyrimidine (SK 5265) or 2,4-diamino-5-(3′,4′-dichlorophenyl)-6-ethylpyrimidine (SK 5266) caused a rapid fall in the total leukocyte count.

The lethal effects of 10 mg/kg, but not of 30 mg/kg, of SK 5265 given intraperitoneally daily for ten injections could be prevented by 30 mg/kg of citrovorum factor given simultaneously. 30 Mg/kg of PGA did not have any antitoxic effect, however. This would seem to indicate that SK 5265 acts on the same metabolic pathway, but at a somewhat different point than A-methopterin.

EFFECTS OF THE N-ETHYLENE SUBSTITUTED PHOSPHORAMIDES ON TRANSPLANTABLE MOUSE LEUKEMIA. Joseph H. Burchenal, S. F. Johnston,* C. Chester Stock, R. P. Parker,* M. L. Crossley, E. Kuh,* and D. R. Seeger.* (Division of Experimental Chemotherapy, Sloan-Kettering Institute for Cancer Research, New York, N.Y., and Research Department of the Calco Chemical Division of the American Cyanamid Company, Bound Brook, N.J.)

It has been demonstrated by Stock *et al.* that certain tri- and diethylene phosphoramides cause an inhibition of growth of Sarcoma 180 in the mouse. For this reason a series of such compounds have been tried out for their activity against mouse leukemia. N,N′,N″-triethylene phosphoramide (SK 3818), N,N-diethyl-N′,N″-diethylene phosphoramide (SK 4614), and N-(3-oxapentamethylene)-N′,N″-diethylenphosphoramide (SK 5623) all caused a significant increase in survival

time in mice inoculated with the A-methopterin-sensitive (Ak4) or resistant (Ak4R) strains of transplantable leukemia when given at the maximum tolerated doses of 5, 15, and 25 mg/kg, respectively, 3 times weekly. Somewhat similar results will be reported with other compounds of this series.

In mice with advanced leukemia of these strains, a marked fall in total leukocyte count from approximately 50,000–100,000 to below 10,000 occurred with 24–48 hours after single doses of these derivatives. *In vivo* sterilization of leukemic cells has also been demonstrated for single intraperitoneal doses at 2–5 times the LD_{50} level.

LINKAGE OF PULMONARY ADENOMAS INDUCED WITH URETHAN. Walter J. Burdette. (Department of Surgery, Louisiana State University, New Orleans, La.)

It is known that pulmonary tumors occur with increased frequency in mice after treatment with carcinogenic hydrocarbons, nitrogen mustard, and urethan. In an effort to determine whether different chemicals act through the same pathway to produce pulmonary adenomas, linkage studies were carried out for tumors induced with urethan and compared to previous studies by Heston, who used 20-methylcholanthrene.

Mice of the W strain, which carries the genes *sh-2*, *wa-2*, and *f*, were mated to the susceptible A strain, which carries the genes *Sh-2*, *Wa-2*, and *F*. Females of F_1 generation were then backcrossed to males of the W strain, and urethan was administered to the resulting offspring. The mice were sacrificed at 28 weeks of age and the number of pulmonary adenomas determined for each individual. The data were then arranged so that the mean number of tumors for the two phenotypes of each pair of allelomorphs, or marker genes, could be compared. In this manner it was found that genes for susceptibility to this method of inducing tumors are linked to *Sh-2* and *Wa-2*, but not to *F*, with no indication that tumor incidence was secondarily related to the genes in question through differences in body weight. Since Heston found that there is linkage to *Sh-2* and *F* but not to *Wa-2* when the carcinogen is used, the mode of tumor induction does not appear to be entirely the same for 20-methylcholanthrene and urethan in the case of pulmonary adenomas.

THE ACID PROFILE OF TUMORS AND NORMAL TISSUES FOLLOWING THE INJECTION OF MALONATE *IN VIVO*. H. Busch* and Van R. Potter. (McArdle Memorial Laboratory, University of Wisconsin, Madison, Wis.)

Anion exchange chromatography of the acidic components of perchloric acid filtrates of tissue samples permits quantitative study of a number of acids including acidic amino acids and acids of the citric acid cycle. Marked changes in these chromatograms—acid profiles—appear following the injection of malonate, including the appearance of large peaks in the malonate position, marked increases in the succinate peak, and variable effects on the amino acid peaks. The initial dose was established at 1.2 ml. of 1 M malonate and was followed in 1 hour by the same dose. One hour after the second injection, the animals were killed, and the tissues were removed, homogenized, and treated with perchloric acid. The increase in titration in the succinate peak corresponded to the following numbers of micromoles per gram of fresh tissue listed: kidney, 7.5; liver, 4.8; thymus, 5.0; heart, 2.2; lung, 2.3; muscle, 0.8; spleen, 1.4; blood, 1.3; brain, 0.5. For the tumors, the following increases were found: Flexner-Jobling, 4.8; Walker 256, 4.0; hepatoma (azo dye-induced), 3.0; Jensen sarcoma, 4.5; papilloma, 2.2. In the controls, the level of titration in the succinate peak was not above 0.02 μM per gram wet weight in tissues other than the kidney, muscle, and liver. Very marked diminution in peaks containing amino acids was noted in the Flexner-Jobling series concomitant with the increase in acidity in the succinate peak.

FORESTOMACH PAPILLOMAS AND GASTRITIS IN THE RAT AFTER TREATMENT WITH 2-AMINOFLUORENE AND DERIVATIVES. Perihan Cambel and F. E. Ray. (Cancer Research Laboratory, University of Florida, Gainesville, Fla.)

2-Aminofluorene and derivatives (2-acetylaminofluorene, benzylaminofluorene, benzoylaminofluorene, and azo derivatives I to VIII) were administered to 277 rats (Sprague-Dawley-Holtzman) by skin painting. 2-Aminofluorene was also administered intraperitoneally in an emulsion. Forestomach papillomas were observed after treatment with 2-aminofluorene, 2-acetylaminofluorene, benzylaminofluorene, benzoylaminofluorene, compounds VI and VIII. The highest incidence occurred with the 2-aminofluorene emulsion. No papillomas were observed in untreated control rats. Gastritis was seen after treatment with the compounds and the solvents (amylacetate, benzene). Hemorrhagic erosions and ulcerations of the glandular stomach were noted after treatment

with most of the compounds. Ulcerations of the forestomach were found after administration of compounds III and V. A lower incidence of hemorrhagic erosions and ulcerations was seen in 23 controls treated with solvents. Cortisone prevented the occurrence of hemorrhagic erosions and ulcerations in 2-acetylaminofluorene-treated rats. No incidence of glandular carcinoma was observed in the total of 367 treated and untreated animals.

Forestomach papilloma induction and hemorrhagic erosions and ulcerations were prevented by cortisone during 2-acetylaminofluorene administration. Occasionally, hemorrhagic erosions were observed in untreated control rats. Fourteen cortisone-treated rats did not show these lesions.

It is concluded that the intraperitoneal administration of a 2-aminofluorene emulsion is best suited for the induction of papillomas of the rat's forestomach. All aminofluorene derivatives produce some form of gastritis accompanied or not by hemorrhagic erosions and ulcerations.

EXPERIMENTAL TERATOGENESIS IN ROOSTERS' TESTES. Rodney Carleton,* Nathan B. Friedman, and E. J. Bomze.* (Division of Laboratories, Cedars of Lebanon Hospital, Los Angeles, and Department of Pathology, University of Southern California School of Medicine, Los Angeles, Calif.)

Teratoid tumors were produced by injecting zinc chloride into roosters' testes. The injected solution caused necrosis of testicular tissue. In surviving tubules at the edges of the necrotic zone the seminiferous epithelium was replaced by undifferentiated elements which formed solid cords. Proliferation of these cells proceeded outside of the tubules and resulted in the formation of monocellular growths. Mixed neoplasms with both undifferentiated and differentiated tissue as well as pure teratomas developed subsequently. These experimentally produced teratomas apparently evolve from undifferentiated tumors not unlike "embryonal carcinomas."

FURTHER STUDIES ON THE NATURE OF THE CHANGE IN POLAROGRAPHICALLY REDUCIBLE SUBSTANCES IN CARCINOGENESIS. C. Carruthers and V. Suntzeff. (Division of Cancer Research, Washington University School of Medicine, St. Louis, Mo.)

Further studies on the nature of polarographically reducible substances, the characteristics of which change during carcinogenesis, have been carried out. Purification has been aided considerably with the use of paper partition chromatography. With this technic the reducible substance has been obtained crystalline from epidermis. This substance melts at 231°–232°C. with decomposition; it has one group at pKá of 3.9 and a second group of pKá 6.03; it contains nitrogen, carbon, oxygen, and hydrogen, and its polarographic behavior and absorption characteristics in the ultraviolet are the same as those previously reported. The reducible substance from squamous-cell carcinomas has different R_f values regardless of the solvent employed, and, although not obtained in crystalline form, its properties are very different from those of the substance from epidermis. These differences are in the half-wave potentials at various pH levels, the absorption characteristics in the ultraviolet, and its solubility.

The reducible substance from mouse, rat, and horse muscle, as obtained with use of paper partition chromatography, is different from that present in the epidermis of the mouse, rat, and man. The differences are in the half-wave potentials at various pH levels, R_f values, and in absorption characteristics in the ultraviolet. Physical measurements on the crystalline substance obtained from muscle suggest that it contains a pyridine ring.

The reducible and ultraviolet absorbing material from tumors of mammary glands, epidermis, muscle, and liver of mice show similar chromatograms, but different from the material from the tissue of origin of the tumors

XYZ FACTORS FOR MOUSE MAMMARY CANCER E 0771. Albert E. Casey, Howard H. Shear,* and Joanne Gunn.* (Laboratories of the Baptist Hospitals, Birmingham, Ala., the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, and the Department of Bacteriology and Immunology, University of Minnesota, Minneapolis, Minn.)

Of 148 matched young adult C57BR/cd mice inoculated subcutaneously by trocar with E 0771 mammary carcinoma (C57BL/6 origin), in three experiments, 59 were controls not previously treated with E 0771 tumor or E 0771 tumor extracts. Ten had been injected once 1–2 weeks prior with 0.2 cc. of lyophilized liver from normal C57BR/cd mice; 9 had been injected once 1–2 weeks prior with 0.2 cc. of lyophilized 15091a tumor tissue; and 40 had received no previous treatment of any sort.

The 89 experimental mice had been injected once subcutaneously in a different site 1–2 weeks prior with 0.1 cc. of one to four saline suspensions

of E 0771 tumor tissue prepared as follows: (*a*) 30 mice with viable E 0771 tumor cells (fifteen with sedimented tumor cells resuspended after centrifugation of the saline suspension for 15 minutes at 1,200 r.p.m. and the supernatant removed); (*b*) twenty mice with supernatant fluid from the saline suspension above prepared by 4 centrifugations at 1,200 r.p.m. for 15 minutes each, the supernatant fluid being transferred to a new tube after each centrifuging; (*c*) nineteen with E 0771 tumor tissue kept frozen 8 days in the CO_2 box, −70°C; (*d*) twenty with lyophilized E 0771 tumor tissue.

Among the 148 mice, 16 died from tumor as follows: 0 of 59 controls (0 per cent); 2 of 30 doubly transplanted with E 0771 (6 per cent); 2 of 19 doubly transplanted, once with frozen E 0771 (11 per cent); 4 of 20 treated with supernatant fluid from fresh E 0771 (20 per cent); 8 of 20 given lyophilized E 0771 (40 per cent).

ACTION OF TESTOSTERONE PROPIONATE ON THE PITUITARY ACTIVITY OF CASTRATED OR X-RAYED FEMALE MICE IN PARABIOSIS WITH NORMAL FEMALES.

C. H. Chang* and Gertrude J. Van Eck* (introduced by W. U. Gardner). (Yale University School of Medicine, New Haven, Conn.)

Hormonal imbalance results in ovarian tumors in gonadectomized animals of several species bearing intrasplenic or intrapancreatic ovarian grafts. Ovarian tumors did not occur when the grafts became adherent to areas drained by the caval system, in animals with one normal gonad, or in those given estrogens or androgens. Estradiol benzoate (16.6 μg. weekly) or testosterone propionate (1.25 mg. weekly) prevented ovarian tumors (Cancer Research, **9**: 35, 1949).

X-rayed mice acquire similar ovarian tumors. One normal ovary or injections with estrogens prevented tumor formation. Testosterone propionate (1.25 μg. weekly) was ineffective (Proc. Soc. Exper. Biol. & Med., **75**: 434, 1950). To determine if testosterone propionate would control hypophyseal gonadotrophin release, the following experiments were undertaken. Young adult female mice of the C57 brown strain were irradiated (175 r) and united in parabiosis each with a normal female; 22 pairs survived. Beginning 2 weeks after the union, the castrated parabionts of one group received four weekly injections of 1.25 mg. testosterone propionate; one, 2.5 mg. testosterone propionate; and one served as control. One week after the last injection the animals were sacrificed. Comparison was made with surgically castrated pairs (30) which were similarly treated. The increase in weight of the ovaries and uteri of the intact partners was prevented by 1.25 or 2.5 mg. testosterone propionate. The overproduction of FSH, as determined by ovarian histology and evident in the control pairs, was missing in the pairs treated with androgen. The continuously cornified vaginal smears and the hypertrophic uteri, occurring in parabiotic females with one partner castrated or x-rayed, disappeared when the castrated parabiont had been treated for about 10 days with androgen.

The dosage of 1.25 mg. testosterone propionate administered in the experiments of Li and Gardner is sufficient to prevent hyperactivity of the pituitary. Factors other than overproduction of gonadotrophins must be responsible in the occurrence of ovarian tumors in x-rayed animals.

THE HISTOGENESIS OF MALIGNANT MIXED TUMORS OF MÜLLERIAN ORIGIN (MIXED MESODERMAL TUMOR OF THE UTERUS).

Wallace H. Clark, Jr.,* William H. Sternberg,* and Robert C. Smith* (introduced by Charles E. Dunlap). (Departments of Pathology and Gynecology, Tulane University of Louisiana and Charity Hospital of Louisiana, New Orleans, La.)

Uterine tumors containing a variety of sarcomatous and carcinomatous elements have been described under a variety of terms, including mixed mesodermal tumor, sarcoma botryoides, and carcinosarcoma. During the 6-year period ending in January, 1952, thirteen such tumors have been seen in the Charity Hospital at New Orleans. Each tumor contained at least two varieties of sarcoma, and ten contained carcinomatous elements as well. The sarcomatous elements included endometrial sarcoma, leiomyosarcoma, rhabdomyosarcoma, fibrosarcoma, and chondrosarcoma. The carcinomatous elements were endometrial adenocarcinoma, papillary cystadenocarcinoma, tubal adenocarcinoma, and epidermoid carcinoma.

Our findings indicate that the tumor arises from derivatives of the Müllerian anlage, most frequently the endometrial stroma. Favoring this view are the following observations:

1. Endometrial stroma closely resembles, histologically, the primitive mesenchyme surrounding the embryonic Müllerian duct.

2. In eleven of the thirteen cases endometrial sarcoma was identified.

3. Tissues heterotopic to the uterus, such as striated muscle and cartilage, frequently appeared to originate in nests of endometrial sarcoma.

4. In the endometrium adjacent to one tumor, nests of rhabdomyoblasts were apparently arising from non-neoplastic endometrial stroma.

5. Bosner and Robson applied 20-methylcholanthrene to the endometria of mice and produced mixed tumors, which resemble histologically the tumors in this series.

6. The epithelial structures of the tumors frequently appeared to differentiate directly from endometrial sarcoma and were histologically compatible with Müllerian origin.

The sarcomatous components include a variety of mesenchymal derivatives. However, unlike true teratomas, the epithelial constituents are apparently limited to Müllerian types of epithelium, a situation analogous to Wilm's tumor of the kidney.

EFFECTS OF 2,4-DIAMINOPYRIMIDINES ON MOUSE SARCOMA 180.

D. A. Clarke,* S. M. Buckley, S. S. Sternberg,* C. C. Stock, C. P. Rhoads, and G. H. Hitchings. (Sloan-Kettering Institute for Cancer Research, New York, and Wellcome Research Laboratories, Tuckahoe, N.Y.

The marked anti-folic property in tests on *L. casei* and antimalarial activity in various species of 5,6-substituted 2,4-diaminopyrimidines suggested their test against mouse Sarcoma 180, one of the animal tumors inhibited by the 4-amino folic acid analogs. The relative anti-folic acid (or anti-citrovorum factor) activities of the 2,4-diaminopyrimidines in various species can be correlated with the chemical nature of the 4- and 6-substituents. Thus, antimalarial activity is exhibited by 5-aryloxy, 5-benzyl, and 5-phenylpyrimidines but is weak or absent among 5-unsubstituted or 5-alkylpyrimidines. Inhibition of Sarcoma 180 has been noted with certain 2,4-diaminopyrimidines with hydrogen or the smaller alkyl groups in the 6-position. The striking biological specificity which is associated with details of chemical structure in this series is illustrated by the 5-(4′-halophenyl) and 5-(5′,4′-dihalophenyl) pyrimidines. Although members of these two series have comparable antimalarial activities, the former are inactive and the latter active as tumor inhibitors. The 2,4-diaminopyrimidines bearing benzyl or phenoxy substitutions in the 5-position have failed to inhibit the tumor.

Attempts to analyze the nature of the antitumor action utilizing natural metabolites to block the effect of the pyrimidine on the tumor will be reported. Cytological changes induced by the active pyrimidines on sarcoma and normal cells will be described.

OXIDATION-REDUCTION STUDIES IN CANCER.

John C. Claudatus* and George T. Lewis. (Medical Research Foundation of Dade County, Miami, Fla.)

A disturbance of the biological equilibrium is, in general, accompanied by a disturbance in the normal oxido-reduction function. The redox potential shows increased negativity, and this is expressed physiologically as tissue asphyxia. Asphyxia is a result of incomplete metabolism, the tissues being flooded with unburned links from the metabolic chains. These links are usually acid in nature, and their accumulation causes a tendency towards acidosis. The action of buffers, however, may result in an increased tissue pH which is optimum for the synthetic activity of the tissue proteases. This latter action is also stimulated by the negative redox potential.

Under the above conditions, the amino acids which are not burned are stored in the form of proteins, thus increasing the protein mass and resulting in a tissue growth. If the change in the environment is not too great, this growth will be benign. A deep-seated change in the milieu results in a malignant growth which has an analogy in the change occasionally observed in fungi growing on an altered nutritive medium.

There will be reported determinations of the redox potential, pH, and the total reducing capacity of specimens of blood and urine from normal individuals and those suffering from cancer.

The above working hypothesis will call for an increased negativity of the redox potential, normal or increased pH values, and an increase in the total reducing capacity of the blood and urine of cancer patients, as compared to that of normal individuals.

A NEW TRANSPLANTABLE RAT TUMOR.

D. H. Copeland and R. W. Engel. (Department of Animal Husbandry and Nutrition, Alabama Polytechnic Institute, Auburn, Ala.)

A mammary carcinoma, originally produced by feeding 2-acetylaminofluorene to a female rat of the AES (Alabama Experiment Station) strain, has been successfully transplanted. This tumor has been maintained through thirteen passages. Initially, the percentage of "takes" was low (15–20 per cent), and the tumor transplant tissue appeared to be completely dormant for periods of 5–10 months before initiating active growth. After two passages from mother to daughter (third and fourth transplants), the tumor initiated active growth within 2 or 3 days after transplanting with

90 to 100 per cent of "takes" in either male or female rats of the AES strain colony.

After thirteen passages the tumor grew at a fairly uniform rate and usually attained sufficient size in 4–5 weeks to cause death of the host.

The microscopic characteristics of the original and early transplants of the tumor will be illustrated. Certain changes that have occurred in the tumor during passage through animals on nutritionally inadequate diets will be discussed.

A MALIGNANT BASALOMA TRANSPLANTABLE IN HAMSTERS. EDWARD D. CRABB and MARGARET A. KELSALL. (Beta Sigma Phi Cancer Research Foundation and Department of Biology, University of Colorado, Boulder, Colo.)

Since extramedullary hematopoiesis in the spleen and periportal infiltration of lymphocytes and plasmacytes into the liver occurred in all hamsters bearing implanted mixed-cell sarcoma for 15 or more days, it was deemed desirable to see if a transplanted carcinoma would induce similar changes.

Tissue implants from six passages of a spontaneous carcinoma (identified by Dr. R. M. Mulligan as a pigmented basal-cell carcinoma) were implanted subpannicularly and/or subcutaneously in the lumbar region of 35 hamsters. Carcinomas having a duration of 57–100 days seldom metastasized. However, of the ten hamsters having a duration of 119–271 days, one had metastasis to lungs only, one to lungs and lymph nodes, and four to nodes only. The livers of 27 hamsters having a tumor duration of 57–271 days showed periportal infiltration of lymphocytes and plasmacytes; while the spleens of 20 of the 27 had hematopoietic foci. The jugular blood of the 20 of which counts were made showed a leukocytosis, due chiefly to increased polymorphonuclear leukocytes and lymphocytes, which was not correlated with the extent of periportal infiltration or splenic hematopoiesis.

CHEMOTHERAPY OF CANCER IN RATS. IV. THE TREATMENT OF TRANSPLANTED CANCERS IN RATS WITH N-PENTAMETHYLENE- AND N-(3-OXAPENTAMETHYLENE)-N′,N″-DIETHYLENEPHOSPHORAMIDES. M. L. CROSSLEY, J. B. ALLISON, R. P. PARKER, E. KUH, and DORIS R. SEEGER. (Bureau of Biological Research, Rutgers University, New Brunswick, N. J., and the Calco Chemical Division of the American Cyanamid Company, Bound Brook, N.J.)

The continued investigation of the ethylenephosphoramides for the treatment of cancer in rats shows that N-pentamethylene-N′,N″-diethylenephosphoramide and N-(3-oxapentamethylene)-N′,N″-diethylenephosphoramide are quite effective in bringing about regressions of Sarcoma 231 in King A rats and Flexner-Jobling carcinoma in Sprague-Dawley rats. It was found previously that N,N′,N″-triethylenephosphoramide could be administered in larger doses than 2,4,6-triethylenimino-*s*-triazine with no demonstrable toxic symptoms. With a dose of 0.2 mg/kg injected twice daily for periods of 15–65 days, the tumors regressed completely in the majority of the rats treated. The N-pentamethylene-N′,N″-diethylenephosphoramide in doses of about 0.4 mg/kg injected twice daily, either intraperitoneally or subcutaneously, produced no demonstrable toxic symptoms and caused complete regressions of both tumors in about 70 per cent of the rats treated. Similar results were obtained with the N-(3-oxapentamethylene)-N′, N″-diethylenephosphoramide, but at higher dose levels.

CHEMOTHERAPY OF CANCER IN RATS. V. LEUKOCYTOSIS ACCOMPANYING THE GROWTH OF TRANSPLANTED TUMORS IN RATS. M. L. CROSSLEY, J. B. ALLISON, and RHODA SLOVIK.* (Bureau of Biological Research, Rutgers University, New Brunswick, N. J.)

In the investigations of the effect of ethylenimines on rat tumors it has been observed that there is a marked leukocytosis accompanying the growth of the tumors. The rate of increase in white blood cells appears to parallel the rate of tumor growth. The leukocytosis is due, essentially, to neutrophilia. In the untreated tumors the neutrophils may be as high as 80 per cent of the white blood cells, while the normal would average between 15 and 20 per cent. When tumor regression takes place there is a reversion of the neutrophil/lymphocyte ratio toward normality. When the tumor fails to respond to treatment the neutrophilia persists. Treatment with high doses of ethylenimine compounds may cause the white blood cell count to fall without corresponding regression of the tumor, but in such cases the abnormal ratio of neutrophils to lymphocytes persists.

CHEMICAL FRACTIONATION OF MAMMALIAN SPERM INTO THREE PARTS: BASIC PROTEIN, NUCLEIC ACID, AND LIPOPROTEIN. R. DUNCAN DALLAM* and

LLOYD E. THOMAS* (introduced by Eugene Roberts). (Department of Biochemistry, University of Missouri, Columbia, Mo.)

Mammalian sperm have been reported by various workers to contain no nucleoprotamine or nucleohistone extractable by the usual means (with water or neutral salt solutions). This finding has been confirmed in the case of bull sperm, but basic protein and nucleic acid, having quite different than usual solubility properties, have been isolated from the heads of these cells. Some data on their characteristics are presented.

A lipoprotein has also been isolated from the heads of bull sperm. This complex contains slightly more than 25 per cent lipid, which includes phospholipid and cholesterol. Some results of electrophoresis and other studies on the nature of the lipoprotein are presented.

The sperm heads can be fractionated, by a simple procedure, into three parts which make up almost all the total substance of the heads. The lipoprotein is obtained by extracting with an alkaline solution (pH 11 or somewhat less alkaline), and the basic protein and nucleic acid by extracting with more strongly alkaline solutions. The lipoprotein precipitates from solution at about pH 6, the basic protein in the region of pH 11, and the nucleic acid at about pH 2.

HEMOANTIBODIES IN MOUSE LEUKEMIA.

ISRAEL DAVIDSOHN and KURT STERN. (Department of Pathology, Chicago Medical School, and Mount Sinai Medical Research Foundation, Chicago, Ill.)

We reported previously that Akm mice free of leukemia showed a low incidence and low titers of natural agglutinins for sheep and chicken erythrocytes, as compared to ten other inbred strains. A lower level of immune antibodies in Akm mice with transmitted leukemia, which were injected with sheep or chicken red blood cells, was also noted. These studies have been extended to other strains and other types of leukemia: AK_4 line of transmitted lymphocytic leukemia in strain Akm; line 1210 of lymphocytic leukemia and methylcholanthrene-induced leukemia in strain DBA; and myeloid leukemia C1498 in strain C57 black. In general, significantly lower titers of immune hemolysins for sheep and chicken red cells were found in mice after development of leukemia. As previously reported, the depressing effect on antibodies was limited to hemolysins. The immunization with red cell antigens did not affect the leukemic process, as judged from hematologic and histologic findings and from growth and survival rates. In contrast to leukemia, presence of various transplanted and induced tumors failed to affect significantly hemoantibodies in mice. The possible significance of this difference in the effect of mouse leukemia and mouse tumors will be discussed.

CYTOLOGICAL STUDIES OF THE RELATIONSHIPS BETWEEN MICRO-ORGANISMS ISOLATED FROM TUMOR TISSUE AND NORMAL AND MALIGNANT CELLS OF THE HOST.

IRENE COREY DILLER. (The Institute for Cancer Research, Philadelphia, Pa.)

For several years past we have been studying the relationship of tumor growth to micro-organisms isolated from tumor tissue. In this field the findings of Gerlach and Wuerthele-Caspe are by far the most consistent yet reported. The organisms isolated by these investigators appear to be identical, at least in their cultural and morphological characteristics. The special contribution of Wuerthele-Caspe is the demonstration of the acid-fast nature of these organisms at some stage of the life history which relates them to the causative agents of tuberculosis and leprosy (mycobacteria). When mouse tumors, including ascites tumors, and blood of mice bearing spontaneous carcinoma, are cultured in the special medium devised by Alexander-Jackson at pH 6.8, organisms apparently identical to those of Wuerthele-Caspe can be isolated. Smears of tumor cells incubated in Jackson's medium and stained with Jackson's stain or with the McManus-Hotchkiss technic show one or more minute acid-fast bodies inside the degenerating tumor cells. From these grow out an amorphous substance within which is formed a delicate mycelium studded with varying numbers of minute acid-fast granules. These can also be recognized in smears of mouse tumors appropriately stained and may represent an intermediate step between bacterial and fungal forms. The organisms are pathogenic to mice in varying degrees. The histological and pathological changes elicited when they are injected into mice of the strain in which the tumor arose at different ages will be discussed.

DIETHYLSTILBESTROL-INDUCED MAMMARY CANCER IN RECIPROCAL F_1 HYBRIDS BETWEEN NEGATIVE AND POSITIVE INBRED LINES OF RATS.

W. F. DUNNING and M. R. CURTIS. (University of Miami, Coral Gables, Fla.)

Strain differences in the incidence of experimentally induced mammary cancer were demon-

strated in rats treated with pellets containing 4–10 mg. of diethylstilbestrol. By this technic it was shown that approximately 80 per cent of the rats of A × C line 9935 that survived for 300 or more days and 63 per cent of the August line 990 rats that survived for 240 or more days developed mammary cancer. No mammary cancers developed in similarly treated rats of Copenhagen line 2331. Except for the exogenous provision of the hormonal stimulation, this situation is analogous to that found in inbred strains of mice with a low and a high incidence of mammary cancer and affords an opportunity to test for the presence or absence of a virus factor in the etiology of induced mammary cancer in rats.

Reciprocal F_1 hybrids were obtained from crosses between rats of Copenhagen line 2331 and A × C line 9935 and between Copenhagen line 2331 and August line 990. Cholesterol pellets containing 4–10 mg. of diethylstilbestrol were implanted subcutaneously in the scapular region of 120 hybrids of the former crosses and 134 hybrids from the latter crosses when the rats were 3–4 months of age.

Data on the incidence of mammary cancer, the average latent period, survival, body weights, and pituitary weights of these hybrids will be discussed.

THE TUMOR-BREAKING PROPERTY OF BACTERIAL POLYSACCHARIDE AND CAPILLARY FRAGILITY. WALTER H. EDDY, BORIS SOKOLOFF, and RITA POWELLA.* (Southern Bio-Research Laboratory, Florida Southern College, Lakeland, Fla.)

Increased capillary fragility is closely associated with the alarm reactions induced by bacterial toxins. Crocker rat carcinoma, average size 7–8 c.mm., was used to demonstrate the role of capillary fragility in the tumor-breaking property of bacterial polysaccharide. In the first series, 25 rats received simultaneously 0.5 mg/100 gm/wt of P-25, Shear bacterial polysaccharide, and 10 mg/100 gm/wt of flavonoids; 66 per cent survived, the rest lived for an average of 46 hours. The control, given P-25 without flavonoids, succumbed in an average of 7 hours, 44 minutes. It is known that flavonoids prevent the increase in capillary fragility induced by bacterial toxins. In the next series, 25 rats bearing Crocker carcinoma were placed on a Sherman-LaMer scorbutogenic diet to which 5 per cent D-gluco-ascorbic acid was added. The ascorbic acid level of blood was brought down to 0.01–0.02 mg/100 cc of plasma, and 0.1 mg/100 gm/wt of P-25 was injected at that time. Mortality rate was 25 per cent, as against 15 per cent of the control group kept on regular diet. The area of tumor destruction was 5.3 times larger than that in the control, and in some instances the destruction was complete. Increased capillary fragility seems to be an important factor in this phenomenon.

NATURAL RESISTANCE AND ACQUIRED IMMUNITY TO TUMORS TRANSPLANTED TO THE ANTERIOR CHAMBER OF HOMOLOGOUS HOSTS. E. J. EICHWALD, H. Y. CHANG,* and M. HATTORI.* (Department of Pathology, University of Utah, College of Medicine, Salt Lake City, Utah.)

To determine the extent to which strain barriers are lowered by use of the anterior chamber as a transplantation site, several mouse tumors were transplanted to various mouse strains. Equal numbers of each strain received subcutaneous or anterior chamber transplants. The barriers of all strains were lowered; establishment of tumors in the anterior chamber occurred in the vast majority of mice of all foreign strains, while this did not take place after subcutaneous inoculation. The extent to which strain barriers were lowered differed with the tumor and the strain. In no instance were strain barriers abolished. When the tumors grew progressively, the survival periods were prolonged, and metastatic dissemination did not occur. However, the tumors often regressed, presumably because of the development of a resistant state. It is therefore surmised that the natural resistance of a foreign host to a tumor is by-passed by use of the anterior chamber but that the barrier of acquired immunity is not overcome. Transplantation to the anterior chamber thereby becomes a convenient means of testing the strength of the immune response of a host to a tumor. Subcutaneous transplantation is less suitable for this purpose, since many tumors fail to become established and therefore cannot demonstrate the development of a resistant state. Administration of cortisone, therefore, does not necessarily increase the incidence of progressive growth of subcutaneously transplanted tumors, while it raises the incidence following anterior chamber transplantation.

ELECTROPHORETIC STUDIES ON THE WATER-SOLUBLE PROTEINS OF LIVER DURING AZO DYE CARCINOGENESIS IN THE RAT. NOREEN ELDREDGE* and J. MURRAY LUCK. (Department of Chemistry, Stanford University, Stanford, Calif.)

The changes occurring in the water-soluble proteins of rat liver during azo dye carcinogenesis

were studied electrophoretically. 3′-Methyl-4-dimethylaminoazobenzene was the carcinogen employed. Studies were carried out on the proteins soluble in water at pH 5 from control and precancerous liver, hepatomas, and blood serum from hepatoma-bearing rats. Electrophoresis experiments were carried out in phosphate buffer, pH 7.7, $\Gamma/2$ 0.1

No significant differences were observed between the electrophoretic diagrams of the extracts from control and precancerous liver; however, the electrophoretic diagrams of the soluble hepatoma proteins were very different from those of liver. While 80 per cent of the proteins of liver had mobilities of -3.0×10^{-5} cm²/volt sec, or less, the majority of the tumor components had mobilities above -3.0 mobility units, the major component having a mobility of -6.08×10^{-5} cm²/volt sec.

The electrophoretic mobilities of the soluble proteins of the hepatoma corresponded to the mobilities of blood serum proteins. However, there was a decrease in the albumin and an increase in the α-globulin in the tumor extracts as compared to blood serum. Studies made in veronal buffer at pH 8.6, $\Gamma/2$ 0.1 revealed that the increase in α-globulin was due to the α_2 component. The electrophoretic diagrams from hepatoma extracts may, therefore, be due entirely or in part to entrapped serum and not to actual tumor proteins.

TUMOR INHIBITION BY 2,4-BIS(ETHYLENIMINO)-6-CHLOROPYRIMIDINE. Gertrude B. Elion, George H. Hitchings, C. Chester Stock, K. Sugiura, and S. M. Buckley. (Wellcome Research Laboratories, Tuckahoe, N.Y., and Division of Experimental Chemotherapy, Sloan-Kettering Institute for Cancer Research, New York, N.Y.)

The marked tumor inhibitory activity of 2,4,6-tris(ethylenimino)-*s*-triazine against various tumors led to the preparation and testing of several pyrimidines containing ethylenimino groups. Studies of 2,4-bis(ethylenimino)-6-chloropyrimidine have revealed its inhibitory activity for Sarcoma 180 and certain other mouse tumors and its greater effectiveness against several rat tumors. The inhibition of the Walker carcino-sarcoma 256 is in agreement with the studies of Hendry *et al.*

INHIBITION OF BROWN-PEARCE CARCINOMA CELLS BY SUSPENSIONS OF LYMPH NODES AND SPLEEN FROM IMMUNE HOSTS. John T. Ellis* and John G. Kidd. (Department of Pathology, The New York Hospital, Cornell Medical Center, New York, N.Y.)

Lymph nodes, spleen, and other tissues, procured from "immune" and control rabbits (defined below), were minced, pressed through a monel metal sieve, and suspended in buffered Ringer's solution enriched with glucose and fresh rabbit serum. The suspensions, containing comparable numbers of individually suspended cells as shown by counts, were mixed with suspensions of Brown-Pearce carcinoma cells and incubated at 37° C. for 2 hours. Those procured from the spleens and lymph nodes of the "immune" rabbits (animals that had overcome Brown-Pearce carcinomas and later resisted implantations with the carcinoma cells) regularly inhibited or abolished the ability of the tumor cells to grow when the mixtures were subsequently implanted into the muscles of susceptible hosts. Under identical conditions, suspensions made from the following tissues had no such effect: (*a*) lymph nodes and spleens from normal rabbits, (*b*) hyperplastic lymph nodes and spleens from rabbits immunized with horse serum, and (*c*) kidney from normal and tumor-immune rabbits. Sera produced from the immune rabbits were devoid of specific Brown-Pearce antibodies (J. Exper. Med., **83:** 227, 1946); furthermore, they did not inhibit growth of the carcinoma cells, nor did suspensions of lymph nodes from normal rabbits in mixture with serum from immune rabbits. The findings confirm and extend previous studies on the mechanism of immunity to transplanted cancer cells (Fed. Proc., **8:** 360, 363, 1949; **9:** 385, 1950), and they provide further evidence that lymphoid cells of immune hosts may act directly against tumor cells *in vitro*.

THE ANALYSIS OF URINARY KETOSTEROIDS IN CANCER PATIENTS BY COUNTER-CURRENT DISTRIBUTION. Lewis L. Engel, Polly C. Olmsted,* and Ira T. Nathanson. (Medical Laboratories of the Collis P. Huntington Memorial Hospital at the Massachusetts General Hospital, and the Department of Biological Chemistry, Harvard Medical School, Boston, Mass.)

Measurement of the partition coefficients of typical ketosteroids in various solvent systems indicated that counter-current distribution would give satisfactory separation of at least the major components of the urinary ketosteroid fraction. With 89–139 transfers in the 50- and 100-tube Craig apparatus, distributions were carried out on the neutral ketonic fractions of cancer patients and suitable control patients. The effects of the administration of various hormonal agents were studied. After analysis of the distribution, char-

acterization of the components of the mixture was made by partition coefficient and by infrared spectroscopy.

The effects of the administration of both dehydroepiandrosterone and testosterone to a woman with metastatic cancer of the breast and also to a woman with rheumatoid arthritis have been studied. Preliminary analyses indicate qualitative as well as quantitative changes in excretion patterns. The patterns in the control periods have been compared to the patterns obtained in normal pre- and postmenopausal women. Distribution of the ketosteroid fraction from the urine of a woman with adreno-genital syndrome gave a pattern strikingly different from that obtained from a control subject. The administration of cortisone was followed by a reduction in ketosteroid excretion and an altered pattern.

INFLUENCE OF STRAIN OF RAT ON LIVER TUMOR PRODUCTION WITH 4-DIMETHYLAMINOAZOBENZENE. R. W. Engel. (Department of Animal Husbandry and Nutrition, Alabama Polytechnic Institute, Auburn, Ala.)

Five female and five male weanling rats each of the AES (Alabama Experiment Station) and the SD (Sprague-Dawley) strains were fed a diet containing 0.045 per cent 4-dimethylaminoazobenzene (DAB) for a period of 4 months. This treatment produced severe gross liver injury in all the AES rats, in contrast to little or no apparent gross liver damage in the rats of the SD strain. Five of the AES rats had liver tumors varying in size from a few mm. to 1.5 cm. The average liver weights, expressed as per cent of body weight, were 7.35 and 4.62 for the AES and the SD rats, respectively.

The more severe hepatotoxic action of DAB in the AES strain was also evident when body weights were compared. Rats of the AES strain made average body weight gains of 35 gm., contrasted with average body weight gains of 85 gm. in the rats of the SD strain during a 12-week period.

Preliminary results indicate that rats of the AES strain have a lower riboflavin requirement than rats of the SD strain.

Liver slices from young adult stock rats of both strains were incubated with DAB in phosphate buffer at pH 7.4.

The capacity for destruction of the dye was more than twice as great in livers of the SD strain than in livers of the AES strain.

BIOSYNTHESIS AND INHIBITION OF PURINES. Morris Engelman,* Horace B. Gillespie,* Leonard Weintraub,* and Samuel Graff. (Department of Biochemistry, Institute of Cancer Research, College of Physicians and Surgeons, and Francis Delafield Hospital, Columbia University, New York, N.Y.)

Numerous experiments have demonstrated that 8-azaguanine is an inhibitor of growth of *Tetrahymena geleii* and also that it is a unique carcinostatic agent. These properties of 8-azaguanine, apparently involving different mechanisms, have stimulated wider investigation. On the one hand the search for more potent or more useful carcinostatic agents has resulted in the synthesis and study of a number of guanine analogs which will be discussed.

Tetrahymena geleii can convert guanine to adenine, whereas the metazoa convert adenine to guanine, suggesting a common intermediate in both processes. It had already been indicated that purine synthesis is probably accomplished through an imidazole intermediate. A hypothetical intermediate which can apparently be converted to either guanine, adenine, or hypoxanthine by biologically possible reactions is 4-formamidino-5-imidazolecarboxamide. The synthesis and biological behavior of this compound will be discussed.

STUDY OF THE REGENERATION PROCESSES IN FISHES AFTER AMPUTATIONS OF DORSAL FINS WITH AND WITHOUT MELANOTIC TUMORS. Recai Ermin* and Myron Gordon. (University of Istanbul, Turkey, and New York Zoölogical Society, New York, N.Y.)

The regeneration of normally and atypically pigmented dorsal fins was studied in several series of platyfish, *Xiphophorus* (*Platypoecilus*) *maculatus* and platyfish hybrids (*X. maculatus-X. helleri*) carrying the macromelanophore gene *Sd*. Some of the hybrids had amelanotic melanomas of the dorsal fin. The fins were amputated. Regeneration of the fin and its pigment cells were studied.

The degree of fin regeneration depends upon the genetic stock of the fish. In normally pigmented fishes the fins and pigment cells regenerate completely. In abnormally pigmented fishes showing melanosis of dorsal fins, after amputation, the fin forms a colorless blastema; then, as the fin continues to regenerate, macromelanophores appear, migrating apparently from the original pigment cells which are located at the base of the fin and from the intermuscular macromelanophores. The

degree of melanosis is restored in the regenerate fin of young hybrids within 3 weeks, but in older specimens melanosis is always less after 6 months than in the original.

Fins of fishes previously having melanomas that are restricted to fin alone rarely regenerate completely. The regenerate fin is usually larger and secondarily develops a melanosis rather than a melanoma. A fish previously having an amelanotic melanoma on the body ventral to the dorsal fin as well as on the fin, develops a blastema and a melanoma of the fin almost simultaneously.

The pigment cells which are involved in the development of melanosis of the dorsal fin do not influence the growth of regenerating fin cells. The pigment cells of a melanoma at the base of the dorsal fin may destroy all or part of the regenerated dorsal fin.

TUMORS IN MONKEYS. FRANK H. J. FIGGE. (Department of Anatomy, University of Maryland Medical School, Baltimore, Md.)

It has been demonstrated by other investigators that spontaneous tumors are infrequent in monkeys and that monkeys are extremely resistant to carcinogenic agents. In the hope of obtaining additional data on the possible co-carcinogenic action of porphyrins, two monkeys were injected in the pectoral region with 5 mg. of methylcholanthrene in sesame oil. In the male monkey, 400 μc. of radon in oil was injected with methylcholanthrene. The female was injected with 4 mg. of hematoporphyrin at a number of sites 2–3 inches from the methylcholanthrene injection site.

Two and ½ years after injection, the female monkey was sacrificed because of a prolapsed rectum. At necropsy, the cervix uterus appeared to be much larger than the normal monkey cervix. Examination revealed a tumor of the dorsal wall of the cervical canal and uterus. The normal polyp-like structure in the cervical canal was not involved, except at its base. The firm hard tissue appeared to be a nonmalignant myoma with hyalin degeneration. However, one of three pieces transplanted to the anterior chamber of a male guinea pig eye grew very slowly and progressively for about 8 months but is now static. Attempts have been made to transplant the tumor to other female monkeys. No tumors were observed in the male monkey. In view of the recent confirmation of co-carcinogenic effects of porphyrins by English investigators, repetition of this work on a larger scale would be desirable.

THE RESPONSE OF TISSUE β-GLUCURONIDASE TO SEX HORMONES IN MICE OF PURE INBRED STRAINS. WILLIAM H. FISHMAN, G. RIOTTON,* M. H. FARMELANT,* and F. HOMBURGER. (Cancer Research and Cancer Control Unit, Tufts College Medical School, Boston, Mass.)

Organs of certain strains of mice have been found to possess characteristically low β-glucuronidase activities (Morrow *et al.*, J. Nat. Cancer Inst., **10:** 657–62, 1949). The present study was undertaken to determine whether or not β-glucuronidase activity in tissues of such mice and of mice belonging to a high glucuronidase strain could be stimulated by the administration of sex hormones.

Accordingly, the tissues (liver, kidney, spleen, seminal vesicles, and uterus) of mice of a low glucuronidase strain (C3H) and of a high glucuronidase strain (129) were studied with respect to the sex of the animal and the effects of castration. In other experiments, androgen and estrogen, either separately or in combination, were given to intact and castrate mice of both strains. The duration of each experiment was 14 days.

A consistent finding was the striking increase in kidney β-glucuronidase which followed androgen administration, unaccompanied by any change in the liver enzyme level. With estrogen administration, a significant increase in liver β-glucuronidase activity occurred with no effect on the kidney enzyme level. β-Glucuronidase is excreted in the urine as a function of the level of renal β-glucuronidase activity induced by androgen administration.

These experiments would appear to demonstrate the interplay of genetic and endocrine factors in the control of β-glucuronidase activity in certain tissues of mice of pure inbred strains.

EFFECT OF 8-AZAGUANINE ON PURINE UTILIZATION BY *TETRAHYMENA GELEII*. MARTIN FLAVIN* (introduced by Dr. Samuel Graff). (Department of Biochemistry, Institute of Cancer Research, College of Physicians and Surgeons, and Francis Delafield Hospital, Columbia University, New York, N.Y.)

This ciliated protozoan, whose growth has been shown by Kidder and co-workers to depend on exogenous guanine and to be markedly inhibited by 8-azaguanine, has previously been found to utilize guanine-8-C^{14} for the biosynthesis of all nucleic acid guanine and adenine and to utilize adenine-8-C^{14}, in the presence of less than half optimal guanine, for the biosynthesis of nucleic acid adenine only.

Since mammalian tumor tissues, which do not

appreciably utilize guanine for nucleic acid biosynthesis, have also in some cases shown growth inhibition by 8-azaguanine, the mechanism of action of this antimetabolite in Tetrahymena is of interest.

Growth inhibition of strain H by 8-azaguanine has now been found to be readily reversible by adenine and hypoxanthine, as well as by guanine, and, since under certain conditions reversal by the former appeared to be noncompetitive whereas reversal by guanine was competitive, experiments were undertaken to determine whether or not a block in the conversion of guanine to adenine was involved in the mechanism of 8-azaguanine inhibition in this organism.

Under the experimental conditions used, such a block was found not to occur. It was further shown that in the presence of optimal guanine and adenine-8-C^{14}, nucleic acid adenine was derived about equally from both.

LYSINE-DEFICIENT AND PHENYLALANINE-DEFICIENT DIETS IN HODGKIN'S DISEASE. Walter J. Frajola,* Lilla S. Wu,* John W. DeVore,* and Herman A. Hoster† (introduced by Hans G. Schlumberger). (Departments of Physiological Chemistry and Medicine, Division of Cancer Research, College of Medicine, The Ohio State University, Columbus 15, Ohio.)

Because the amount of protein synthesis and growth is determined by the availability of that essential substance present in the least amount, restriction of the dietary intake of an essential amino acid should result in the restriction of protein synthesis and/or a retardation of tumor growth. A dietary regime for diseased individuals which makes possible a study of the effects of restrictions of essential amino acids is described. The amino acids in amounts recommended by Rose (Fed. Proc., **8**: 546, 1949) are fed as water mixtures together with a cornstarch pudding and "cake." Deficiencies are produced by the removal of the acid from the mixture. Studies of five patients with Hodgkin's disease are reported. Nitrogen balance is used as the criterion of the adequacy of the diet. Positive nitrogen balance was achieved with the eight essential amino acids and added glycine. Restriction of phenylalanine or of lysine resulted in negative nitrogen balance. Chemical analyses included total nitrogen, creatinine, uric acid, and 17-ketosteroid determinations. Clinical studies included physical examination for enlarged nodes, x-ray studies, sedimentation rate, and blood and temperature analyses. Indications of some possible therapeutic effects will be described.

† Deceased May 14, 1951.

INFLUENCE OF COPPER-DEFICIENT AND MAGNESIUM-DEFICIENT DIETS ON SUSCEPTIBILITY OF MOUSE SARCOMA TO X-RAY. W. R. Franks, A. McGregor,* M. M. Shaw,* and J. Skublics.* (Banting and Best Department of Medical Research, University of Toronto, Toronto, Canada.)

The influence of certain diets which might play a role in the susceptibility of tumor to x-ray has been studied. A copper-free diet was first investigated, because of the known fact that the activity of tissue catalase is thereby reduced. This in turn might be expected to reduce the rate of removal of any peroxide formed by the irradiation. Mice transplanted with Sarcoma 37, after 4 weeks on the copper-deficient diet, showed a diminished rate of tumor growth and an increased susceptibility to x-ray, as shown by an increased number of tumor regressions. The depression of the liver catalase from the diet was found to be decreased further by the Sarcoma 37 tumor and by the x-ray.

Similarly, a low magnesium diet was studied, since magnesium probably plays a role as coenzyme in phosphorylating mechanisms in tumor; these systems have been shown to be susceptible to x-ray. Sarcoma 37 transplanted into magnesium-deficient mice likewise grew slower than in controls. However, the effect of x-ray on the tumor was not increased; in fact, there was significantly less inhibition in tumor growth following the x-ray treatment. Similarly, the number of regressions was significantly less in the magnesium-deficient mice whose tumors were treated with x-ray. This is in contrast to the effect of the copper-deficient diet. These results would suggest that the effect of x-ray on tumor is increased when the body catalase is decreased and, conversely, is decreased when phosphorylating mechanisms are inhibited.

THE EFFECTS OF NITROFURANS ON THE NORMAL TESTIS AND ON TESTICULAR TUMORS (SEMINOMA). Charles E. Friedgood,* Anthony L. Danza,* and Anthony Boccabella* (introduced by C. Carruthers). (Department of Surgical Research, Maimonides Hospital, Brooklyn, and

the State University of New York College of Medicine, New York, N.Y.)

Nitrofurazone has been reported to have an inhibitory effect upon the growth of sarcoma S-13 in C3H mice. Upon post mortem examination of these tumor-bearing animals, marked testicular degeneration was observed. It was decided to determine whether or not this effect could be reproduced in adult albino rats. Furacin and two derivatives, furadroxyl and furadantin, were used in this study. Of the three, furacin was found to cause the greatest degeneration, which was reversed in 1 month after cessation of treatment.

Since the testicular effect of these compounds on rats was known, it was decided to assess their effects on human patients with prostatic carcinoma. Six such patients were treated, and a marked testicular atrophy was noted, with little or no direct effect on the prostatic carcinoma. Since a profound cytotoxic action was observed on the normal testis, it was decided to treat malignant tumors of the testis. Four cases of seminoma have been treated. In all these there has been marked degeneration of the tumor cells, increased fibrosis, and decreased cellularity of the tumor. This effect of the nitrofurans on malignancies of the testes warrants further investigation.

COMBINATION CANCER CHEMOTHERAPY WITH 8-AZAGUANINE AND A RIBOFLAVIN ANTAGONIST. Ruth A. Fugmann* and Daniel M. Shapiro. (Department of Surgery, College of Physicians and Surgeons, Columbia University, New York, N.Y.)

This laboratory is engaged in studies on the effects of vitamin antagonists, alone and in combination with other carcinostatic agents, on neoplastic growth. Recently, data on the enhancement of the inhibitory effect produced by 8-azaguanine when used in combination with desoxypyridoxine, a B_6 antagonist, were presented for the mammary adenocarcinoma 755 grown in C57 mice.

In continuation of these studies, the effect of flavotin, an anti-riboflavin compound, was evaluated upon this same mouse breast cancer. Flavotin, either alone or in combination with 8-azaguanine, inhibited the growth of the tumor in every experiment. Statistical analysis demonstrates that the difference between the mean tumor weights of the control and treated animals is significant in almost every instance.

It appears possible that these studies will lead to far more effective chemotherapy with 8-azaguanine.

CONDITIONS OF TRANSPLANTATION AND HORMONAL SECRETIONS OF PITUITARY TUMORS INDUCED BY I^{131}. J. Furth, W. T. Burnett, Jr.,* E. Gadsden,* and J. N. Dent.* (Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn.)

Tumor-like growths induced in the pituitary by I^{131} are readily transplantable in similarly treated hosts and metastasize to regional lymph nodes, but only one of five strains of tumors so established by successive transplantations in I^{131}-treated hosts proved transplantable in normal hosts. Tadpole assays and morphological changes in hosts bearing such tumor grafts gave evidence of the secretion of thyroid-simulating and gonadal hormones. The dependency of pituitary growth on the lack of thyroid function is indicated by the failure of the tumors to grow in normal hosts, with the exception of one strain, and by the atrophy of the tumors following administration of thyroid hormone. A puzzling change in all hosts bearing large tumors is hyperplasia of extrahepatic biliary ducts, notably of the ampulla, and a tremendous cystic dilation of these ducts. There are no data in the literature relating pituitary hormones directly to the biliary tract. This carcinogenesis is a clear example of how lack of a hormone leads to proliferation of cells responsible for the maintenance of that hormone and how stimulation leads to conditioned and occasionally to autonomous growth.

STUDIES ON URINARY THIOCYANATE EXCRETION FROM ADMINISTERED MALONONITRILES. Emery M. Gal, E. Swirnofsky,* and David M. Greenberg. (Division of Biochemistry, University of California, School of Medicine, Berkeley, Calif.)

The detoxification mechanism in the body of the substituted malononitriles was studied by determining the daily excretion of thiocyanate in the urine of normal and Walker carcinoma-bearing rats for 6 days after the intraperitoneal injection of the nitriles. The excretion of endogenous thiocyanate in the controls was 72γ per day, as against 39γ for the tumor-bearing rats. There was no difference in the conversion of administered cyanide to thiocyanate between normal and tumor-bearing animals. With the nitriles, on the other hand, the amount of thiocyanate was decreased in the tumor-bearing animals, with certain nitriles by as much as 50 per cent. *p*-Nitrobenzal- and 5-nitrofuranalmalononitrile yield no thiocyanate in either normal or tumor-bearing

animals. The excretion of thiocyanate from cyanide-producing nitriles was not influenced by either sodium azide or 5-nitrofuranalmalononitrile. Iodoacetate and, particularly, 2,4-dinitrophenol accelerated the rate and the amount of the thiocyanate excreted. The resistance of nitro-substituted malononitriles to formation of cyanide seems to be different from that associated with the biological properties of dinitrophenol. Evidently, the enzyme system responsible for releasing cyanide from nitriles can be interfered with *in vivo*.

CHICK LIVER ABNORMALITIES CAUSED BY YOLK SAC-GROWN VIABLE TUMORS AND BY DEAD TUMOR AND DEAD ABNORMAL LIVER MATERIAL INJECTED INTO THE EGG YOLK SAC.

Irving Galinsky and C. L. Spurr. (Carnegie Institution of Washington, Dept. of Genetics, Cold Spring Harbor, L.I., and Baylor University College of Medicine, Houston, Texas.)

A mammary carcinoma from a DBA mouse was grown continuously in fertile eggs for 23 generations. This tumor produced abnormal livers (green, green and yellow, red and yellow) in the embryos of most of the eggs in which it was grown. The tumor and abnormal livers were killed by slow freezing at −10° C. and thawing at 28° C. and then were macerated, diluted with saline, and injected into the yolk sac in exactly the same manner as the live tumor. Dead material produced no growth when injected into eggs or mice, and microscopic examination showed the cells to be ruptured. Tissues injected into control eggs were taken from eggs in which nothing had been injected or grown.

A total of 920 living embryos was examined (12 days after egg inoculation) in eighteen different experiments. In every case there is a statistically significant increase in the number of abnormal livers found in eggs injected with live tumor, dead tumor, and dead green livers from tumor eggs. There is no significant increase in the number of abnormal livers in eggs injected with saline, dead normal livers, and dead green livers from normal eggs. Dead tumors and dead green livers from tumor eggs cause about the same per cent increase in abnormal livers but not as great as that caused by the live tumor.

FURTHER OBSERVATIONS ON OVARIAN TUMORIGENESIS IN TRANSPLANTED OVARIES.

W. U. Gardner. (Yale University School of Medicine, New Haven, Conn.)

The appearance of ovarian tumors in intrasplenic ovarian grafts in castrated rats and mice has been observed in several laboratories, and considerable evidence has been accumulated to involve the pituitary gonadotrophic hormones in their etiology. The tumors appear some time after 6 months. The following experiment was undertaken to determine the fate of the tumors arising in intrasplenic grafts in castrated mice and to compare the changes in ovarian grafts in other sites.

Mice of the BC strain or its hybrids (BC × C3H) were used. The grafts were made into mice 32–64 days of age. The donors were of the same age or from mice as old as 520 days. Some mice received transplants in the sacro spinales muscles, and in some males the ovaries were transplanted in the testes. The animals were examined repeatedly during the course of the experiment to determine the time of appearance of palpable tumors, and the tumors were followed until the animals died or death was imminent. Several were followed for more than 1 year after palpable nodules first appeared in the spleen. Twenty-seven of 47 gonadectomized mice receiving intrasplenic grafts had ovarian tumors at from 280 to 719 days; five nontumorous mice had grafts adherent to the body wall, four grafts could not be found, and eleven grafts were nontumorous (276–726 days). Seven of thirteen ovarian grafts from donors 273–520 days of age were tumorous at 520–717 days.

The tumors were granulosa-cell tumors, mixed granulosa-cell tumors, and tubular adenomas, one embryoma, and one teratomatous growth. Subcutaneous or intratubular grafts had follicles in 700-day-old hosts. One embryoma occurred in a testis bearing an ovarian graft.

TISSUE CULTURE STUDIES OF THE PROLIFERATIVE CAPACITY OF CERVICAL CARCINOMA AND NORMAL EPITHELIUM.

George O. Gey, Ward D. Coffman,* and Mary T. Kubicek.* (Departments of Surgery and Gynecology, Johns Hopkins Hospital and University, Baltimore 5, Md.)

This is a report of an evaluation *in vitro* of the growth potential of normal, early intra-epithelial, and invasive carcinoma from a series of cases of cervical carcinoma. Comparable cytological and tissue culture studies were actually carried out on selected biopsies of normal and neoplastic areas of the same cervix. Thus far, only one strain of epidermoid carcinoma has been established and grown in continuous roller tube cultures for almost a year. It grows well in a composite medium of chicken plasma, bovine embryo extract, and human placental cord serum. The autologous nor-

mal prototype is most difficult to maintain under comparable cultural conditions. Most of the tissue from other cases showed rapid keratinization of the cells grown in cultures whether from normal or neoplastic areas. Some of the hormonal aspects of the problem will be discussed.

ALTERATION OF THE GROWTH RESPONSE OF TISSUE CELLS *IN VITRO* IN SERUM FROM PARTIALLY HEPATECTOMIZED RATS. André D. Glinos and George O. Gey. (Division of Cell Physiology, Department of Surgery, Johns Hopkins Hospital and University, Baltimore 5, Md.)

Fourty-four male adult rats were operated on in pairs, a partial hepatectomy being performed on one of the animals and a sham operation simulating partial hepatectomy on the other. Twenty-four hours after the operation the animals were bled. The sera obtained from the hepatectomized and the control animal of each pair were then compared with respect to their action on the growth of tissue cells *in vitro*. Primary liver explants from young rats 3 weeks of age, as well as a normal fibroblastic strain, were used as test tissues. Initiation of outgrowth, organization and morphology of the colonies, and duration of growth period were used as criteria for the evaluation of the action of these sera on the cultures. It was found that blood serum from the hepatectomized animals was able to enhance significantly the growth of both the primary liver explants and the strain of fibroblasts. Quantitative data on the duration of the growth period at varying concentrations of sera from hepatectomized and control animals were obtained. The significance of these findings with respect to the nature of the alteration of the serum induced by partial hepatectomy and its meaning for liver regeneration will be discussed.

FACTORS CONCERNING RADIOSENSITIVITY AND "IMMUNIZING ABILITY" OF MOUSE MAMMARY TUMORS. Anna Goldfeder and Fusaye Inouye.* (Cancer Research Laboratory, New York City Department of Hospitals and New York University, New York, N.Y.)

Previous investigations demonstrated a significant difference in the rate of growth, metabolic activity, and radiosensitivity of two mouse mammary adenocarcinomas designated C3H and dbrB. In addition, grafts of either tumor, attenuated with specific doses of x-radiation, rendered mice of the respective strains immune. This immune state was detected when viable tumor grafts failed to grow upon implantation into these mice.

Studies of the above mentioned characteristics were extended to two highly inbred lines of mice of the C3H and DBA strains. Both lines bear the milk agent, and both have a spontaneous tumor incidence of over 90 per cent.

A spontaneous mammary tumor which arose in a mouse of Bittner's high cancer line was excised, and small fragments were implanted into mice of the same line. A latent period of 12–14 days was established with 100 per cent "takes." A dose of 5,500 r prevented the growth of tumor grafts upon implantation into autogenous hosts, and smaller doses of x-radiation yielded variable percentages of "takes." Doses of about 10,000 r destroyed tumors *in situ* of about 1.5 cm. in diameter.

Identical experiments are being carried out simultaneously on DBA mice bearing the milk agent and on fostered C3H mice without the milk agent.

Those mice in which irradiated implants failed to produce tumors or in which the tumors regressed following irradiation are being tested for their immune state.

Results of these experiments will be discussed in relation to the mechanisms involved in induced resistance to malignant growth.

COMPARATIVE EFFECT OF HIGH AND LOW VOLTAGE X-RADIATION ON BIOLOGIC MATERIALS. L. Whittington Gorham, Leonard Clark,* Donald Remp,* and Kenneth B. Olson.* (Union College, Department of Biology; Albany Medical College, Department of Medicine, Division of Oncology, Albany, N.Y.)

The comparative effects of high (50 Mev) and low (124 kv.) voltage x-radiation has been studied. In biologic material maximum ionization occurs at the surface or point of entrance with a beam of low voltage radiation and at a point 4–5 cm. beyond the point of entrance with high voltage radiation. Young adult male rats were anesthetized lightly with ether and placed in cylindrical, plastic containers. The tails were pulled outside and deflected in a constant manner. The rats were thus considered to be a solid cylinder of tissue and placed within a pressed wood phantom. Radiation was administered along the length of the animals in a tail-to-head direction. With an average body dose of 900 r, half of 63 animals survived 14 days with 124-kv. radiation, while half of 23 animals survived only 3 days after a similar dose from a high voltage emitting source (50

Mev). This indicates a significant difference in effect. Studies of blood, bone marrow, liver, spleen, kidney, and testes were made at various intervals after both types of radiation. A comparison of the ratio of weights of spleen and testes to total body weights was made. These organs were selected because the testes are superficial and near the point of entrance of the beam and the spleen is at about the point of maximum ionization with high voltage radiation. Catalase determinations were made on liver and spleen.

SELECTIVE UPTAKE OF RADIOACTIVE SULFUR BY HUMAN CHONDROSARCOMAS. RAYMOND G. GOTTSCHALK and HERBERT C. ALLEN, JR.* (Radioisotope and Medical Research Units and Laboratory Service, Veterans Administration Hospital, and Departments of Pathology and of Medicine, Baylor University College of Medicine, Houston, Texas.)

The ability of cartilage of rats to retain sulfur and to incorporate it into chondroitin sulfate and the frequent similarity of metabolism of homologous normal and malignant tissues suggested that radioactive sulfur might be taken up by chondrosarcomas. Tracer doses of S^{35} were given as sodium sulfate to two adult patients. One had a chondrosarcoma of the ilium with multiple metastases; tissues were obtained by biopsy after 65 hours and at necropsy 11 days after the injection. The other patient with a slowly growing chondrosarcoma of the tibia received S^{35} 50 hours prior to amputation. Radiation counts on the exposed surfaces of the tissues, radioautographs, and determinations of activity of the total sulfur, isolated as barium sulfate, indicated about the same degree of concentration of S^{35} in normal cartilage, in the chondrosarcomas, and in the metastases; the amount of tracer was lower in the viscera and soft tissues. The concentration fell rapidly in the blood, while the cartilaginous tissues retained high levels of S^{35}. These findings will be discussed in relation to possible diagnostic or therapeutic applications.

THE USE OF THE BRAIN AS A TRANSPLANTATION SITE. HARRY S. N. GREENE. (Yale University School of Medicine, New Haven, Conn.)

The ability of the brain to support the growth of homologous and heterologous tissues has been investigated. Adult, embryonic, and cancer tissues grow on homologous transfer, while benign tumors and precancerous tissues fail to survive, and heterologous transfer is successful only in the case of embryonic tissue and cancer. The superiority of the brain over the anterior chamber of the eye as a nidus for the growth of human cancer will be discussed.

PRELIMINARY OBSERVATIONS FOLLOWING THE ADMINISTRATION OF α-PELTATIN TO PATIENTS WITH NEOPLASTIC DISEASE. E. M. GREENSPAN,* J. COLSKY,* E. B. SCHOENBACH,* and M. J. SHEAR. (Clinical Research Unit of the National Cancer Institute, United States Public Health Service Hospital, Baltimore, the Department of Preventive Medicine, Johns Hopkins University Medical School, Baltimore, and the Laboratory of Chemical Pharmacology, National Cancer Institute, Bethesda, Md.)

Since 1947 when α-peltatin was isolated from crude podophyllin at the National Cancer Institute, it was found to have a structural formula closely related to that of podophyllotoxin, and various workers there have shown that it induces damage in a variety of animal tumors; they have also studied its pharmacological effects and the histopathological changes induced in normal tissues.

In view of these observations, the response of 45 patients with carcinoma or lymphoma to the administration of α-peltatin was investigated. The compound was injected intravenously in doses of 0.1–0.5 mg/kg of body weight. A single dose greater than 0.15 mg/kg in the lymphoma group or 0.25 mg/kg in the carcinoma group frequently induced chill, fever, nausea, vomiting, and diarrhea 2–6 hours after injection. Patients received one to twelve injections of α-peltatin at 48–72 hour intervals. One patient developed a transient peripheral neuropathy, and several noted paresthesias in the hands and feet. No evidence of hematopoetic, hepatic, renal, cardiovascular, or cerebral dysfunction attributable to treatment was observed.

Two instances of diffuse necrosis of ulcerated squamous carcinoma and several instances of a rapid transient regression in splenomegaly and lymphadenopathy in patients with leukemia, Hodgkin's disease, lymphosarcoma, and giant follicular lymphosarcoma, after the administration of α-peltatin, are reported. These changes were observed only after administration of doses which produced some or all of the toxic reactions noted. The interpretation of histologic findings in biopsied tumor tissue after treatment was equivocal. With the exception of one patient with giant fol-

licular lymphosarcoma, the administration of α-peltatin did not appear to influence significantly the clinical course of the patients observed in this study. Despite the objective changes noted, there appeared to be no definitive improvement in the clinical status of the patients.

Although the consistently reproducible gross and histologic alterations induced by much higher doses of α-peltatin in animal tumors were not paralleled in this preliminary clinical experience, the observations suggest that further study of the mechanism of action of this compound is warranted.

THE LIVER NUCLEIC ACID UPTAKE OF C^{14} ADENINE DURING AZO DYE CARCINOGENESIS. A. Clark Griffin, William E. Davis,* and Marie O. Tifft.* (Department of Biochemistry, Stanford University, Stanford, Calif.)

Adenine-8-C^{14} was injected intraperitoneally into normal rats, rats fed diets containing 3′-methyl-4-dimethylaminoazobenzene, and rats with liver tumors. Animals from each group were sacrificed 3 hours, 24 hours, and 7 days following administration of the isotope. The liver and tumors were fractionated into acid-soluble, lipid-soluble, total nucleic acid, desoxyribonucleic acid (DNA), and pentosenucleic acid (PNA) components. Nuclei prepared from the tissues were similarly fractionated. Phosphorus and C^{14} were determined on all fractions; nitrogen and DNA were determined on appropriate fractions.

The isotope activity in the liver tissues was greatest 3 hours after administration and was reduced by 30 and 50 per cent at 24 hours and 7 days, respectively. Tumor tissue contained less of the isotope at all time intervals than liver tissues. The DNA fraction of normal liver incorporated little of the labeled adenine. DNA from the liver of rats fed azo dye accounted for 5, 10, and 15 per cent of the C^{14} activity of the total tissue nucleic acid at the 3-hour, 24-hour, and 7-day intervals, respectively. Comparable DNA values for tumor tissues were 16, 25, and 38 per cent, respectively. The nuclear PNA incorporated a large amount of C^{14}. This relatively small component of the cell, presumably of nucleolar origin, appears to play a major role in the cell proliferation, development, and metabolism. Three hours, 24 hours, and 7 days after administration of labeled adenine the nuclear PNA of the liver and tumor tissues contained 45, 35, and 25 per cent, respectively, of the C^{14} activity of the total tissue nucleic acids.

SPHERICAL PARTICLES IN FILTERED MOUSE LEUKEMIA EXTRACTS. Ludwik Gross,* Kenneth S. McCarty,* and Irvin J. Cohen* (with technical assistance of Anita E. Posselt*). (Cancer Research Unit, Veterans Administration Hospital, Bronx 68, N.Y.)

Cell suspensions prepared from liver, spleen, and lymphatic tumors of 72 leukemic Ak mice (36 with spontaneous and the remaining with transplanted leukemia) were centrifuged at 3,000 r.p.m. (1,800 *g*) for 10 minutes, and the supernatant was then passed through a Seitz filter, under vacuum pressure of 20 mm. mercury. The ST-3 pads used (Hercules Corporation, Patterson, N.J.) were made from Arizona asbestos and were nonacid-treated; the filters, with the mounted pads, had been autoclaved, prior to their use, at 15 lbs., 115° C., for 1½–2 hours.

Electron microscopic examination of the 72 filtered leukemic extracts revealed in all instances the presence of large numbers of spherical particles varying in diameter from 20 to 200 mμ; the great majority of them, however, were of a larger size, 100–200 mμ; these particles had a smooth surface and high density to the electron beam; they appeared to be attached to the isolated asbestos fibers, separated from the pads in the course of filtration. The asbestos fibers, with the attached spherical particles, could be conveniently visualized by the following procedure: 1 ml. of the filtered leukemic extract was removed carefully from the bottom of the tube with a tuberculin syringe, poured on the top of 2 ml. of triple-distilled water into a celluloid tube, and centrifuged; the initial speed (500 *g*) was raised slowly over a period of 4 minutes to a top speed of 25,000 *g*, and the motor was turned off after an additional minute. The asbestos fibers, with the attached particles, were thus deposited by the centrifugal force directly on the electron grid placed on the bottom of the tube; the particles, having passed through the layer of distilled water, were at the same time washed from interfering proteins and from sodium chloride crystals.

Spherical particles of similar size could also be visualized in centrifugated (not filtered) leukemic samples segregated by electrophoresis.

In five individual experiments, the filtered leukemic extracts containing the particles were inoculated into newborn C3H(f) infant mice less than 12 hours old; as a result, 8 of the 25 inoculated mice developed leukemia at an average age of 8.5 months (L. Gross, Ann. N.Y. Acad. Sc., 1952 [in press]).

The following control experiments were per-

formed: (*a*) Filtered extracts were prepared from normal organs (liver, spleen, kidneys, heart, lungs) of sixteen normal, young, healthy Ak mice; two of them were found to contain spherical particles similar to those found in leukemic extracts; seven additional samples contained spherical particles of a smaller size (20–100 mμ in diameter) only, and possibly also of lesser density to the electron beam, lined along the asbestos fibers; the remaining seven samples did not reveal the presence of particles attached to the fibers. (*b*) Filtered extracts were prepared from organs of 56 normal, healthy foster-nursed C3H(f) mice; nine of them contained large particles, six the small particles, and 41 (73 per cent) did not contain any particles attached to the fibers. (*c*) Of 31 filtered extracts prepared from organs of normal, healthy C57 (black) mice, five contained the large and four the small particles, and 22 (71 per cent) extracts did not contain any particles attached to the fibers. (*d*) Filtered extracts were prepared from ten embryos removed aseptically from healthy, pregnant C3H female mice; three of them contained the large and four the small particles, and in three instances the fibers were free from particles.

FAILURE OF PLACENTA TO PASS RADIOACTIVE GOLD COLLOID. M. L. Haigler* and G. Z. Williams. (Department of Oncology, Medical College of Virginia, Richmond, Va.)

Pregnant rats, rabbits, and dogs in the last third of gestation were injected by the intravenous or intercardiac route with radioactive gold colloid. Large tracer and therapeutic doses were used to test the ability of the placenta to act as a barrier to the colloidal particles of gold. At varying intervals after injection of the sol, animals were sacrificed and the uteri removed intact. The amniotic fluid was obtained and the fetuses removed without contamination by the blood or tissue juices of the mother. No radioactivity could be demonstrated in the amniotic fluid or in the tissues of the fetuses.

CLARIFICATION OF DIFFERENCES IN RADIOSENSITIVITY OF TUMORS IRRADIATED *IN VITRO* AND *IN VIVO* ON THE BASIS OF THE EFFECT OF OXYGEN ON RADIOSENSITIVITY. B. Vincent Hall,* Katherine Hamilton,* and Austin M. Brues. (Division of Biological and Medical Research, Argonne National Laboratory, Chicago, Ill., and Department of Zoölogy, University of Illinois, Urbana, Ill.)

Quantitative radiosensitivity measurements of a transplantable mouse carcinoma were made by measuring the viability and growth of implants irradiated under standardized conditions *in vitro* with x-rays (200 kv. 15 ma; 2 mm. Al filter; dose rate, 1,030 r/min; t.d., 12.7 cm.). Severe depression of their oxygen utilization by cold (0° C.) and cyanide (0.01 M) increased their radiosensitivity (from an LD_{50} near 3,000 r to below 1,500 r), with atmospheric oxygen present. In pure nitrogen, these agents did not affect radiosensitivity. Controlled experiments showed that the radiosensitivity of small tumor fragments was greater than that of larger fragments taken from the same tumor, when irradiated *in vitro* with oxygen present. The *in vivo* radiosensitivity of week-old tumors, well vascularized and rapidly growing in the dorsum of a mouse's foot, which were irradiated without exposing the remainder of the host to radiation, was found to be approximately equal to that of tumor fragments irradiated *in vitro* in atmospheric oxygen with their oxygen utilization severely depressed by cold or cyanide. All the observed radiosensitivities were consistent with the assumed oxygen content of the tumor fragments *in vitro* and intact tumors *in vivo*, as estimated by the Krogh formula $d_o \propto \sqrt{\frac{8C_oD}{A}}$, relating the depth of oxygen diffusion into tissues directly to a diffusion constant and to the environmental oxygen tension, and inversely to the metabolic rate.

Although other factors may be involved as well, it appears possible to account for many of the peculiarities in radiosensitivity of tumors and tumor fragments on the basis of tissue oxygen.

PRESSURE EFFECTS OF INTRACRANIAL NEOPLASMS. Béla Halpert and William S. Fields.* (Veterans Administration Hospital and Baylor University College of Medicine, Houston, Tex.)

The brain is enclosed in an unyielding box with stiff partitions for its major subdivisions; increased pressure in one compartment is gradually transmitted to the others. Thus, an expanding neoplasm within the cranial cavity that results in intracranial hypertension produces structural changes in the brain substance adjacent to and distant from the site of involvement. There occurs an increase in weight of the brain, impairment of blood supply, interference with circulation of cerebrospinal fluid, distortion of adjacent ventricles, and bulging into nearby compartments with pressure grooves and herniations.

The effects of intracranial hypertension on the

brain substance produced by intracranial neoplasms, primary and metastatic, were studied in 40 patients, twenty of whom had primary and twenty had metastatic growths. When the neoplasm was located in the cerebrum, usually there was flattening of the convolutions, narrowing of the sulci, distortion and displacement of the lateral and third ventricles, tentorial pressure grooves, tentorial herniations, herniation under the falx cerebri, and pressure cone over the cerebellum. When located within the midbrain, pons, or cerebellum, obstructing the aqueduct of Sylvius or the fourth ventricle, the neoplasm caused internal hydrocephalus, i.e., symmetrical dilatation of the third and lateral ventricles. Slowly growing extra-axial neoplasms such as hypophysial adenoma, craniopharyngioma, and meningioma produced compression of the nearby brain substance and changes in adjacent parts of the cranium. Three of the primary intracranial neoplasms were multiple or multicentric. Almost all metastatic growths were multiple.

LIPIDS AS THE CAUSATIVE AGENTS FOR THE ABNORMAL PROPERTIES OF THE SERUM ALBUMINS IN HUMAN CANCER.

MARTIN E. HANKE* and HERBERT KAHN.* (Department of Biochemistry, the University of Chicago, Chicago, Ill.)

Certain abnormal properties of serum albumin frequently observed in cancer (decreased coagulability, solubility, and combining capacity for fatty acids) are due, not to changes in the proteins per se, but to the presence in cancer sera of characteristic abnormal lipids which combine with the albumin. Although the fraction of the albumin which is soluble in 38.5 gm. per cent $(NH_4)_2SO_4$ at pH 7.2 is usually smaller in cancer than in normal sera (about 15 and 25 per cent of the total albumin, respectively), lipid-free preparations of albumin from these sera show no such difference in their solubility. The addition to normal serum of certain lipid fractions from cancer sera and from cancer tissue (obtained by differential solubility in acetone and methanol and progressive elution from alumina with petroleum ether, benzene, and diethyl ether) consistently caused a decrease in the solubility of the albumin in 36–41 gm. per cent $(NH_4)_2SO_4$, while corresponding lipid fractions from noncancer sera or normal tissue had no such effect. Spectrophotometry of these lipid fractions from cancer sera showed much more absorption at 270 mμ and less at 450 or 600 mμ than that from normal sera. These lipids are only a part of many substances, normal and abnormal metabolites and drugs, which combine with serum albumin and determine the latter's physical properties. We believe that study of the characteristic lipids of cancer sera and cancer tissue may lead to more reliable laboratory tests for cancer and should reveal something fundamental about its chemical pathology.

RELATIONSHIP BETWEEN CHROMOSOME PLOIDY AND HISTOCOMPATIBILITY IN MOUSE ASCITES TUMORS.

T. S. HAUSCHKA. (Institute for Cancer Research and Lankenau Hospital Research Institute, Philadelphia, Pa.)

Seven suitable ascites tumors have been investigated by Levan and Hauschka (1952) with regard to chromosome number distribution and were found to be characterized by typical ploidy patterns as follows: The DBA thymoma, TA3 mammary adenocarcinoma, and 6C3HED lymphosarcoma had predominantly diploid cell populations. The MC1A rhabdomyosarcoma, Krebs 2 anaplastic carcinoma, Ehrlich carcinoma, and Sarcoma 37 were typified by tetraploid modes, besides numerous aneuploid cells.

Transplantation tests of these tumors in five inbred strains and one heterozygous stock of mice revealed a possible correlation between chromosome number and host-specificity. The three near-diploid ascites survived only in the strains in which they originated, or in F_1, F_2, and backcross hybrids carrying the required dominant histocompatibility genes. Three of the four neoplasms with tetraploid modes grew in all strains of mice. MC1A rhabdomyosarcoma gave inconclusive results.

It is not yet clear whether the increased growth capacity in foreign hosts is brought about by simple polyploidy or by aneuploidy. Heteroploid nuclei could, conceivably, have lost antigens through elimination of chromosomes carrying H-loci; or the immunogenetic reactivity of a cancer cell depends on a definite balance in its entire chromosome set.

The histories of the tumors used in this study have been varied; hence, comparison of histocompatibility in relation to ploidy is not sufficiently critical. More reliable evidence is being sought through inducing heteroploidy in one of the host-specific diploid tumors and through further transplantation tests with distinct clonal sublines isolated from the cell population.

THE DEPHOSPHORYLATION OF ADENOSINE TRIPHOSPHATE BY TUMORS.

LISELOTTE HECHT* and ALEX B. NOVIKOFF.

(Departments of Pathology and Oncology and of Biochemistry, University of Vermont, College of Medicine, Burlington, Vt.)

Last year we reported (Cancer Research, **11:** 273, 1951) that rat tumor homogenate differed from normal liver homogenate in that ATP dephosphorylation was stimulated by calcium as well as by magnesium ions. It was therefore of interest to determine whether different enzymatic reactions were involved.

The rat tumors used in these studies were primary liver tumors, a transplantable liver tumor, and the Walker 256 carcinosarcoma. All were shown to possess considerable adenylate kinase activity. Homogenates, in 0.88 M sucrose, were fractionated by differential centrifugation. Some adenylate kinase activity was found in all fractions. The microsome fraction, which is the most active of the isolated fractions in ATP dephosphorylation, had very little adenylate kinase activity. The supernatant fluid, which possessed a small portion of the ATP-dephosphorylating activity of the homogenate, possessed the major portion of its adenylate kinase activity.

The appearance of adenosine diphosphate (ADP) and 5′-adenylic acid (A5′P), as well as that of orthophosphate, was followed when homogenates and isolated intracellular fractions were incubated with ATP in the presence of $MgCl_2$ and $CaCl_2$. Considerable ADP formation was noted with the liver tumors but not with the Walker carcinosarcoma. In all tumors A5′P appeared and was slowly dephosphorylated.

These results will be compared to those described for normal liver (J. Biol. Chem., **194:** 153, 1952).

TEST FOR A MATERNAL INFLUENCE IN THE DEVELOPMENT OF MAMMARY GLAND TUMORS IN AGENT-FREE STRAIN C3Hb MICE.

W. E. Heston and Margaret K. Deringer.* (National Cancer Institute, Bethesda, Md.)

In strain C3Hb, developed from a litter taken from a high-tumor strain C3H mother by cesarean section and foster-nursed upon a low-tumor strain C57 black female without the mammary tumor agent, 38 per cent of the breeding females have developed mammary carcinomas, although the agent could not be demonstrated.

As a further test for the agent or other maternal influences in the development of the tumors in this strain, reciprocal hybrids were produced from crosses with C57 black. The hybrid females were bred extensively. Of 89 BC3Hb F_1 females (C57 black mothers), six developed mammary carcinoma at an average age of 22 months, and 83 died without mammary carcinoma at an average age of 26.8 months. Of 93 C3HbB F_1 females (C3Hb mothers), four have developed mammary carcinoma at an average age of 22 months, four have had mammary tumors that have not yet been sectioned and some of which may be sarcomas at an average age of 29.5 months, 74 have died without mammary carcinoma at an average age of 26.3 months, and ten are living tumor-free at an average age of 29.8 months beyond which it is doubtful that mammary carcinoma will develop. There was, thus, no evidence of any nongenetic maternal influence received from the C3Hb mothers. The mammary carcinomas of these hybrids, as well as those of the C3Hb females, were apparently the result of the genotype, together with a strong breeding factor in the absence of the mammary tumor agent.

INHIBITION OF SKIN CARCINOGENESIS IN MICE BY MIXTURES OF STRONG CARCINOGENS.

Willard T. Hill,* D. Warren Stanger,* Anthony Pizzo,* Byron Riegel, and William B. Wartman. (Departments of Pathology and Chemistry, Northwestern University, Chicago and Evanston, Ill.)

It has been shown that production of skin tumors in mice by a strong chemical carcinogen is partly inhibited when it is applied in a mixture with one of several hydrocarbons. The claim has been made that the inhibitory power of certain of these inhibitors is related to their being weak carcinogens. It was decided, therefore, to test the effect of mixtures of two strong carcinogens which differed in respect to the latent periods of the tumors they produced when tested separately.

Groups of 30 CAF_1 hybrid mice were painted twice weekly with acetone solutions of the carcinogens. In each experiment one group received a single carcinogen, and a second group received a mixture of the same carcinogen and another carcinogen. The concentration of the second carcinogen was twice that of the first. The two groups were then compared with respect to the mean latent period of tumor production and to the mean yield of tumors per mouse.

Under these conditions, application of a mixture of 9,10-dimethyl-1,2-benzanthracene with 1,2,5,6-dibenzanthracene, with benzpyrene, or with methylcholanthrene resulted in tumors having a mean latent period longer than tumors produced by 9,10-dimethyl-1,2-benzanthracene alone. This

result was most striking when 1,2,5,6-dibenzanthracene was the additional carcinogen. Mixture of this compound with methylcholanthrene resulted also in a mean latent period longer than that resulting when methylcholanthrene was applied alone. No effect on tumor yield per mouse was observed.

SOME BIOLOGICAL AND CLINICAL PROPERTIES OF METHYLANDROSTENEDIOL. F. HOMBURGER, G. RIOTTON,* S. C. KASDON,* C. D. BONNER,* R. M. DART,* and W. H. FISHMAN. (Cancer Research and Cancer Control Unit, Tufts College Medical School, Boston, Mass.)

Experimental studies in mice receiving methylandrostenediol (17α methyl-Δ^5-androstene 3β, 17β-diol) have shown that it is a renotropic, androgenic, and protein anabolic compound and that these effects are obtainable in mice by oral as well as by parenteral administration. This compound also caused a significant reduction in the size of the adrenal glands.

Clinical investigation yielded quantitative evidence of a protein anabolic effect with depression of urinary 17-ketosteroid excretion.

In long-term studies on 50 patients with inoperable cancer of the breast, palliative results equivalent to those expected of testosterone were obtained, accompanied by fewer and less pronounced undesirable side effects.

Our experience with this group of patients, which includes detailed observations of plasma protein, serum calcium, phosphorus, and alkaline phosphatase will be discussed.

THE DEVELOPMENT OF INTERSTITIAL-CELL TUMORS OF THE TESTES IN EXPERIMENTALLY CRYPTORCHID BAGG ALBINO C MICE BEARING GRAFTED OVARIES. ROBERT A. HUSEBY and JOHN J. BITTNER. (Department of Pathology and the Division of Cancer Biology of the Department of Physiology, University of Minnesota Medical School, Minneapolis, Minn.)

Interstitial-cell tumors of the testes occur in several strains of mice following the prolonged administration of large doses of estrogenic hormones. Smelser has shown that ovaries grafted to male guinea pigs that have been made cryptorchid produce goodly amounts of estrogen at the same time that the cryptorchid testes produce rather normal amounts of androgen. Experiments in this laboratory indicate that the same situation obtains in mice. To see if such "physiological" amounts of estrogen in the absence of any considerable degree of interstitial-cell hypofunction would influence interstitial-cell tumor development, ten Bagg albino C males were made cryptorchid when approximately 1 month of age and were immediately grafted with two ovaries from C donors. Six of these males developed large interstitial-cell tumors, and in two others small but macroscopically evident tumors were found. The tumors were noted when the mice were from 17 to 23 months of age, and in all instances they were histologically similar to those reported in heavily estrogenized mice.

As controls sixteen C males were made cryptorchid only. Of these, two developed typical interstitial-cell tumors. In three others microscopic foci of interstitial cells measuring up to a millimeter in diameter were seen, but it was felt that these probably did not represent early centers of neoplasia. No grossly discernible testicular tumors were found by Dr. Kirschbaum in autopsying 86 normal C males 17 months of age or older that descended from the same line but had been housed in his colony.

STUDIES ON THE MECHANISMS OF RESISTANCE TO A-METHOPTERIN IN *STREPTOCOCCUS FAECALIS* AND *LEUCONOSTOC CITROVORUM*. DORRIS J. HUTCHISON,* MARGARET KENNEDY,* and JOSEPH H. BURCHENAL. (Chemotherapy Service, Memorial Cancer Center, and The Division of Experimental Chemotherapy, Sloan-Kettering Institute, New York, N.Y.)

Since the development of resistance to 4-amino-N^{10}-methyl pteroylglutamic acid has been reported in human leukemia, mouse leukemia, and, more recently, in *Streptococcus faecalis* (ATCC 8043), further studies have been made on the resistance of *S. faecalis* and *Leuconostoc citrovorum* to this compound. The resistant organism, *S. faecalis*/A, is capable of substituting certain PGA analogs for PGA, whereas the parent culture is not. Some of these analogs are those that have one of the following modifications in the PGA molecule: N^{10}-methyl, 4-amino, 4-amino-N^{10}-methyl, and 4-amino with the replacement of glutamic acid moiety by aspartic acid, alanine, threonine, or amino malonic acid. However, when there was a 9-methyl substitution in the presence of a 4-amino group, there was no PGA-like activity as in 4-amino-9-methyl PGA and 4-amino-9,10-dimethyl PGA. This resistant organism still has an absolute requirement for PGA or citrovorum factor.

A culture of *Leuconostoc citrovorum* that is some 2,000 times more resistant to A-methopterin than was the parent culture has been obtained from *L. citrovorum* (ATCC 8081) through serial transfers in liquid media containing the citrovorum factor and 4-amino-N^{10}-methyl PGA (A-methopterin). The nutritional requirements of this strain will be discussed. Further studies are in progress with both resistant cultures.

THE BEHAVIOR OF EMBRYONIC CHICK HEART FIBROBLASTS IN SOME PROTEIN-FREE MEDIA.

Aa. Bohus Jensen (introduced by Stanley P. Reimann). (Institute for Cancer Research, Philadelphia, Pa.)

Attempts were made to maintain and grow fibroblasts in the synthetic medium devised by P. R. White, in ultrafiltrates from horse and chicken serum and in embryo juice dialysate, the mitotic frequency being used as a rough criterion for the amount of growth obtained.

Fresh explants of 10-day chick embryo hearts were placed on perforated cellophane in Carrel flasks in 1 cc. of the medium (pH between 7.4 and 7.8), which was changed every 2–3 days. The mitoses and resting nuclei were counted in the zones of migrating cells which developed around the explants.

Three groups of experiments were carried out:

1. Cell colonies were kept in White's medium 14–29 days, then fixed and stained 24–50 hours after the last change of medium. The number of mitoses was found to be about two/5,000 cells, which indicated that practically no growth is taking place under these circumstances.

2. Cell colonies were kept 15–24 days in White's medium, which was then discarded, and chicken or horse serum ultrafiltrate was added. Twenty-four to 72 hours later the cells were fixed and stained, and the mitotic frequency was found to be of the order of magnitude of fifteen mitoses/5,000 cells.

3. Cell colonies which had been kept in White's medium for 20 days were exposed for 24 hours to a medium consisting of equal parts of White's medium and embryo juice dialysate. The mitotic frequency was found to be about 40/5,000 cells.

While growth is evidently not taking place in White's medium and in the serum ultrafiltrates, the somewhat higher mitotic frequency in the presence of embryo juice dialysate may indicate a slow growth in this medium.

No fat droplets were formed in the cells, and the size of the explants and the migration zones had decreased considerably after 38–52 days in all these media. The bearing of the observations reported here on problems of cellular nutrition will be discussed.

AN EVALUATION OF THE SERUM POLYSACCHARIDE AND THE LEAST COAGULABLE PROTEIN CONCENTRATION OF SERUM IN CANCER DETECTION CENTERS.

Ralph Jones, Jr., Otto Rosenthal, Jeannette McC. Shorey, Robert D. Sullivan,* Peggy Beatty,* and Charlotte Witmer.* (C. Willard Robinson Section, Department of Medicine and The Harrison Department of Surgical Research, University of Pennsylvania, Philadelphia, Pa.)

In a study designed to evaluate the possible usefulness in cancer detection centers of nonspecific laboratory methods which are frequently abnormal in patients with cancer, we have measured the tryptophan-perchloric acid reaction (serum polysaccharide of Seibert), and the least coagulable protein concentration of serum (as described by Huggins *et al.*) in 1,796 individuals, at the time they were seen in a cancer detection center.

A parallel study of these tests in patients with proved malignancy and a variety of nonmalignant diseases has been conducted. The tryptophan-perchloric reaction was abnormal in 78 per cent and the least coagulable protein concentration was abnormal in 75 per cent of patients with proved malignancy. Both methods were frequently abnormal in a variety of nonmalignant diseases but were rarely abnormal in healthy individuals.

In patients from the cancer detection centers, one or both tests were abnormal in 238 individuals (12 per cent), at the time of initial testing. In 85 per cent of these patients the determinations were repeated 1 or more times, and the abnormality was found to be consistently present in 74 (4 per cent). These individuals have been subject to detailed study and have been followed for 6–12 months after the abnormality was detected. A comparison has been made of the frequency with which significant disease occurred in this group of patients and in a larger group of patients in whom both tests were repeatedly normal.

Results suggest that these tests are rarely abnormal in the absence of significant disease, but the frequency with which unsuspected cancer was detected was not increased by the use of these methods.

THE EFFECT OF PRIOR INJECTIONS OF LYOPHILIZED MOUSE TISSUES ON THE SURVIVAL OF NORMAL TISSUE

HOMOIOGRAFTS IN MICE. NATHAN KALISS and DAVID M. SPAIN.* (Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, and the Department of Laboratories and Research of Westchester County, Valhalla, N.Y.)

Tissue grafts from mice of one inbred line to those of an unrelated line (homoiografts) usually regress. However, under certain conditions prior injections of lyophilized mouse tissues lead to a significant number of "takes" of *tumor* homoiografts. In the present instance, inbred mice treated in this manner received grafts of either normal mouse skin, subcutaneous implants of embryonic tissue, or spleen. The mice with embryo and spleen grafts were autopsied 3 months after implantation.

The skin grafts did not survive. There appeared to be a difference in the embryonic tissue grafts in the treated hosts, as compared to the controls, with survival in several of the treated mice of bone marrow, muscle, connective tissue, nerve, and cartilage. Several of the control mice had surviving cartilage. There was a marked inflammatory reaction around the grafts in the controls. In two experiments with spleen grafts from A/Lilly to A/Lilly mice, there was a definite increase in Experiment 1 in the number of animals (both males and females) with surviving grafts in the experimental group (6 of 23 mice) as compared with the controls (0/12; level of statistical significance just over 5 per cent). In Experiment 2, 12/30 treated males had grafts as against 0/10 in the controls (P value <2 per cent). There was no statistical difference in the females (18/57 experimental; 3/18 controls). (It is known to us, from other types of data, that the A/Lilly stock is not genetically homogeneous.)

RELATION OF REGION AND VOLUME OF SHIELDED TISSUE TO PROTECTION AGAINST LYMPHOMA DEVELOPMENT IN IRRADIATED C57 BLACK MICE. HENRY S. KAPLAN and MARY B. BROWN. (Department of Radiology, Stanford University School of Medicine, San Francisco, Calif.)

A significant decrease in lymphoid tumor incidence occurs when a lead shield is placed over the hind leg during x-radiation of C57 black mice (H. S. Kaplan and M. B. Brown, Cancer Research, 11: 261–62, 1951). The present experiment was undertaken to determine whether the degree of protection is related to either the volume or region of tissue shielded. One-month-old C57 black mice, in groups of about 50 each, were given four doses of 168 r each at 4-day intervals. Lead shields covered the following regions during irradiation of the respective groups: one thigh, one entire lower extremity, both lower extremities, the lower abdomen, or the tail. Another group was shielded over alternate lower extremities during each exposure. Two groups were initially shielded over both lower extremities and the tail, and then re-irradiated, either immediately or 24 hours later, with the remainder of the body shielded. Two control irradiated groups received whole-body exposure without shielding; one of these was immobilized during treatment in the same manner as the shielded groups.

Lymphoid tumor incidence was markedly reduced by shielding over the thigh, entire extremity, or lower abdomen, but not affected by shielding of the tail. The protection afforded by shielding both lower extremities or the lower abdomen was somewhat greater than for one extremity or the thigh, although the differences are of borderline significance. The inhibitory effect of shielding the lower extremities was abolished by local irradiation of the previously shielded regions within 24 hours.

LACK OF EFFECT OF THYMIC IMPLANTS ON LYMPHOMA INCIDENCE IN THYMECTOMIZED IRRADIATED C57 BLACK MICE. HENRY S. KAPLAN, MARY B. BROWN,* and CARL W. WALSER, JR.* (Department of Radiology, Stanford University School of Medicine, San Francisco, Calif.)

Thymectomy greatly reduces the incidence of lymphomas in spontaneously susceptible strains of mice (J. Furth, J. Gerontol., **1**:46–54, 1946) and in irradiated C57 black mice (H. S. Kaplan, J. Nat. Cancer Inst., **11**:83–90, 1950). Since most of these tumors arise in the thymus, it had been thought that thymectomy acted simply by removing the susceptible cells of origin. It was recently reported, however, that lymphoma incidence could be restored to usual spontaneous or carcinogen-induced levels in thymectomized mice by subcutaneous implantation of homologous or autologous thymic grafts (L. W. Law and J. H. Miller, J. Nat Cancer Inst., **11**: 253–62, 425–38, 1950), suggesting that the thymus was necessary for "full expression" of the leukemic process, even though the grafts were often uninvolved by tumor.

This result has not occurred with radiation-induced lymphomas of C57 black mice. To date, no lymphomas have occurred among thymectomized irradiated animals, whether or not thymic grafts, either untreated or irradiated *in vitro*, were im-

planted subcutaneously after completion of treatment. Lymphoma incidence has attained expected levels in sham-operated, irradiated animals, and is not significantly different in those receiving thymic grafts than in irradiated controls.

INCORPORATION OF RADIOACTIVE PHOSPHATE INTO TUMOR PARTICLES. E. P. KENNEDY* and H. G. WILLIAMS-ASHMAN* (introduced by Charles Huggins). (Ben May Laboratory for Cancer Research and the Department of Biochemistry, University of Chicago, Chicago, Ill.)

Under conditions suitable for oxidative phosphorylation, extensive incorporation of P^{32}-labeled inorganic ortho-phosphate takes place into the phospholipid, nucleic acid, and "phosphoprotein" fractions of the acid-insoluble residues of tumor cytoplasmic particles as well as into the pyrophosphate groups of ATP. These incorporations are dependent upon the addition of substrate and are sensitive to 2:4-dinitrophenol in the same way as the oxidative phosphorylation of adenine nucleotides. The specific activity of the "phosphoprotein" fraction was the greatest and that of the phospholipid fraction of the acid-insoluble residue the least in experiments 20 minutes in duration. The intense dephosphorylation of adenine nucleotides by tumor particles necessitates the addition of fluoride for extensive incorporation of P^{32} into both the acid-soluble and the acid-insoluble fractions. The kinetics of these incorporations will be discussed.

A COMPARISON OF THE ENZYMATIC ACTIVITY OF RAT AND MOUSE LIVER TUMORS. C. J. KENSLER, M. RUDDEN,* and H. LANGEMANN.* (Department of Pharmacology, Cornell University Medical College, New York, N.Y.)

Choline oxidase activity has been found to be low or absent from rat liver tumors, and this, in conjunction with other evidence, led to the suggestion (Cancer Research, **11**: 264, 1951) that an interference with choline metabolism and transmethylation reactions may play an important part in the production of liver tumors by the carcinogenic azo dyes. Consequently, it appeared desirable to measure the activity of this enzyme and dimethylthetin-homocysteine transmethylase in a variety of liver tumors. Azo dye destruction was measured, as the rat tumors were found not to metabolize these dyes in contrast to normal liver. Succinoxidase, cholinesterase, and tributyrinase activities were also measured. The tumors compared with primary azo dye-induced rat liver tumors, transplanted mouse hepatoma 98/15 (obtained from Dr. G. Hogeboom), transplanted mouse tumor 112B (obtained from Hogeboom), and a transplanted azo dye-induced mouse hepatoma obtained from Dr. Sugiura. In contrast to the negative findings in the rat tumors, the mouse hepatomas contained 15, 33, and 50 per cent of the choline oxidase activity of normal mouse liver. Dimethylthetin-homocysteine transmethylase activity was low or absent in all tumors. Cholinesterase and tributyrinase activity varied from 10 to 120 per cent and succinoxidase activity from 15 to 50 per cent of normal liver. In contrast to the rat azo dye tumors, two of the mouse hepatomas destroyed more dye *in vitro* than did normal mouse liver. The patterns of enzyme activity for the systems measured were characteristic for each tumor. Betaine-homocysteine transmethylase activity was also found to be low or absent in the rat tumors examined and indicates that in azo dye carcinogenesis several enzymes concerned in "labile" methyl metabolism are grossly depleted.

THE EFFECT OF DIFFERENT ESTROGENS ON THE INDUCTION OF RENAL TUMORS AND HEPATOMAS IN MALE GOLDEN HAMSTERS. HADLEY KIRKMAN (introduced by A. Clark Griffin). (Department of Anatomy, Stanford University School of Medicine, Stanford, Calif.)

Sixty-five of 85 male hamsters received subcutaneous implants of pellets of various estrogens. The remaining twenty were retained as untreated controls. Data concerning these animals are given in the following formulae, in which the sequence is as follows: estrogen employed, average duration of treatment in days, average total absorption of estrogen in mg., average age in days, average body weight in gm., average weight of left testis in mg., average weight of hypophysis in mg., percentage of group developing renal tumors, percentage of group developing renal tumor metastases, percentage of group developing hepatomas.

(*a*) controls-0-0-415-123-1453-3.5-0-0-0;
(*b*) diethylstilbestrol-338-31-394-96-82-42.0-100-55-0;
(*c*) alpha estradiol-355-24-406-84-69-16-93-47-0;
(*d*) ethinyl estradiol-345-57-394-92-64-19-0-0-27;
(*e*) Fenocylin-282-47-331-108-731-6-0-0-20.

It is evident that renal tumor formation follows the administration of either diethylstilbestrol or estradiol but does not follow either ethinyl estradiol or Fenocylin. While the incidence of hepato-

mas is much below that of renal tumors, hepatomas occur only in the ethinyl estradiol and Fenocylin groups.

These data suggest that the oncogenic agent responsible for the induction of renal tumors is not the active estrogen but some conjugated breakdown product of it; hepatomas, on the other hand, do not appear to be induced by such a conjugate. Experiments are under way to test this hypothesis.

INDUCTION OF LEUKEMIA IN MICE BY ESTROGENIC HORMONE, METHYLCHOLANTHRENE, AND X-RAYS. ARTHUR KIRSCHBAUM, JOYCE R. SHAPIRO,* and HARRY W. MIXER.* (Department of Anatomy, College of Medicine, University of Illinois, and Departments of Anatomy and Radiology, University of Minnesota, Minneapolis, Minn.)

Estrogenic hormone, methylcholanthrene, and x-rays were used alone and in combination to induce leukemia in the C (Bagg albino), CBA, and DBA-212 strains. Treatment was usually begun at 42 days of age; 3,400 animals of 70 experimental groups are surveyed.

Significant findings included: (*a*) X-rays and estrogen were strongly synergistic in all strains. (*b*) In only DBA mice were methylcholanthrene and estrogen synergistic—the DBA strain was the only one susceptible to independent action of methylcholanthrene. (*c*) Thymectomy produced a disease largely thymic in origin. (*d*) Partial body radiation (thymus only, or all but thymus) with 200 r gave a synergistic effect in C but not CBA strain; although the thymus was not radiated, disease originated in this organ, indicating a "secondary" effect. (*e*) After a single whole-body exposure to 200 r of x-rays a systemic leukemogenic disease with no thymic enlargement appeared late in life; it was similar to spontaneous disease of strains C and CBA. (*f*) One μg. of estradiol dipropionate (EDP) weekly for 14 weeks gave a strong synergistic response with 200 r of x-rays in C mice; 100 r of x-rays strongly synergistic with 5 μg. weekly of EDP. (*g*) A significant synergistic effect was obtained when 1.0 mg. pellets were implanted for from 10 to 40 days. (*h*) A weekly dose of EDP (5 μg.) which was of only threshold leukemogenic effect if begun at 42 days of age was decidedly leukemogenic in C mice if begun at 18 days.

FURTHER STUDIES ON THE MECHANISM OF ASCITES TUMOR GROWTH. GEORGE KLEIN* and EVA KLEIN* (introduced by T. S. Hauschka). (Karolinska Institutet, Stockholm, Sweden.)

Previous investigations on ascites tumor growth (Exper. Cell Res., **2**: 518, 1951) have been continued and now comprise serial experiments with 42 different mouse tumors. Intense tumor cell proliferation in ascites was found to occur regularly only after injection of high cell numbers of certain rapidly growing tumors and, as a rule, only after a number of intraperitoneal exudate transfers. The intensity of this growth was found to be inversely related to inflammatory reaction, solid tumor growth, and survival time of the animals. All slowly growing neoplasms tested gave negative results. Chemical determinations of DNA in ascites cell populations with known mitotic rate and where chromosome ploidy has been determined by Hauschka and Levan (1951) indicated cellular DNA duplication to occur in connection with mitosis, and not gradually during interphase. Colchicine experiments showed that this duplication did not happen until metaphase, at least when tested under the effect of this drug.

LUNG ADENOMAS IN OFFSPRING FOLLOWING INJECTION OF PREGNANT MICE WITH URETHAN. MICHAEL KLEIN. (Cancer Research Laboratory, University of Florida, Gainesville, Fla.)

Pregnant albino mice related to strain A were injected intraperitoneally with a 10 per cent solution of urethan 19 days following the discovery of a vaginal plug. In one group, the mice were allowed to litter and nurse their young. In another, the pregnant females were sacrificed 1 hour and 5 hours following injection. The young were removed by caesarean, exposed ½ hour to 95 per cent oxygen-5 per cent carbon dioxide, and fostered on untreated mothers of the same strain. Six months thereafter, injected mothers and young were sacrificed, their lungs fixed, and tumors counted under a magnification of 12×.

A tumor incidence of 95.9 per cent and an average of 9.4 tumors per lung were observed among the offspring of mothers which had been permitted to litter. All the mothers bore tumors, an average of 9.2 per lung. A tumor incidence of 97.1 per cent was observed among the caesarean-delivered mice which had been exposed to the carcinogen *in utero* for 5 hours. In this group, the young averaged 16.4 tumors per lung. Where the mice were exposed *in utero* for 1 hour, tumor incidence was 100 per cent with an average of 19.1 tumors per lung. Among untreated 6-month-old control mice of the same strain, a tumor incidence of 7.9 per cent and an average of 0.1 tumor per lung were observed. The mechanism of lung tumorigenesis is discussed in the light of these observations.

NEW ULTRAMICROMETHODS FOR ENZYMATIC CYTOCHEMISTRY.

M. J. Kopac. (Department of Biology, Graduate School of Arts and Science, New York University, New York 3, N.Y.)

Enzymatic activities of small cytoplasmic masses may be determined with (*a*) an improved microdilatometric method (Linderstrøm-Lang) and (*b*) the new volumetric ultramicropipettes (Kopac and Harris), which permit the extraction of measured volumes of cytoplasm from living cells.

Certain enzyme-substrate reactions, including the dipeptidases, proteinases, phosphatases, and nucleic acid depolymerases, produce volume changes (electrostriction effects) which can be measured dilatometrically. In reaction volumes of 0.01 c.mm., the hydrolysis of 0.000,001 μM of alanylglycine is measurable—an amount hydrolyzed, for example, within 15 minutes by 1,000 μ^3 of amoeba cytoplasm.

Living amoebas, ascites tumor, free liver, or other isolated cells, when properly supported by pyknotic cushions, may be centrifuged at high speeds without flattening, so that all cytoplasmic inclusions become displaced and rearranged, in accordance with their densities, to form strata. With this procedure, layers of living cytoplasmic matrix, uncontaminated by subcellular particulates (nuclei, mitochondria, etc.), are obtained from which measured volumes can be extracted with the volumetric ultramicropipettes.

A small volume (0.001–0.01 c.mm.) of buffered substrate medium is placed into a drop of non-aqueous gradient fluid. With one of the ultramicropipettes, the cytoplasmic matrix (1,000–10,000 μ^3) is transferred from a centrifuged cell to the substrate mixture. Known amounts of activators, co-substrates, or inhibitors are added with the second ultramicropipette. The reaction mixture is then placed into the gradient column, where density-change-rates are measured. Activities, calculated from these data, are expressed as μM of substrate/hour/1,000 μ^3 of cytoplasm.

Similarly, measured volumes of cytoplasm may be used as substrates for ATP-ase with or without myokinase, nucleic acid depolymerases with or without proteinases, and for other enzymes.

The association of certain enzymes with either the cytoplasmic matrix (submicroscopic particulates) or with the larger subcellular particulates may now be established unequivocally, since there is no irreversible disorganization of cytoplasmic structures prior to the separation of the matrix fraction from the larger particulate fractions.

ISOLATION OF CELL PARTICLES.

Richard Krakaur,* Ada M. Graff,* and Samuel Graff. (Laboratories of the Joseph and Helen Yeamans Levy Foundation, Beth Israel Hospital, and the Department of Biochemistry, Institute of Cancer Research, College of Physicians and Surgeons, and Francis Delafield Hospital, Columbia University, New York, N.Y.)

Recent investigations have emphasized the role of individual cellular components in enzymatic reactions. Many of the enzyme systems and all the nucleic acids, for example, are found, not in uniform solution throughout the cell, but bound in discrete cellular particles.

Differential centrifugation has been employed often for the separation of these particles, notably by Claude, Schneider, and Potter. Selection of particle type was usually accomplished by adjustment of both the magnitude of the applied centrifugal force and the time of application of this force, either one of which is difficult to manipulate.

This communication, however, describes a systematic procedure by which many, if not all, the cellular components may be isolated from a single preparation in good yield without gross impairment of the particles. A tissue homogenate in appropriate medium is centrifuged after the specific gravity of the medium has been adjusted relatively to that of the desired particle. A centrifugal force is applied over sufficient time for the attainment of equilibrium between particle and medium. Only particles of specific gravity *higher* than that of the medium sediment out. It is necessary to utilize a medium which can be adjusted over the required ranges and which does not impair either the morphological, chemical, or functional integrity of the particles. Very strong sucrose solutions are almost ideal for this purpose.

Morphological, enzymatic, and chemical evidence on the fractions isolated from strong sucrose media are presented.

SEPARATION OF RAT LIVER MITOCHONDRIA INTO TWO MORPHOLOGICALLY AND BIOCHEMICALLY DISTINCT SUBFRACTIONS.

Anna Kane Laird,* Oddvar Nygaard,* and Hans Ris* (introduced by H. P. Rusch). (McArdle Memorial Laboratory and Department of Zoölogy, University of Wisconsin, Madison, Wis.)

The large granule fraction of normal rat liver, sedimented at 18,000 × *g*. from 0.88 M sucrose homogenates, forms a densely packed lower layer and a more loosely packed "fluffy" upper layer.

These layers have been separated by successive washings and resedimentations. The more densely packed material is characterized by a very low ratio of pentosenucleic acid to protein nitrogen, varying from $\frac{1}{5}$ to $\frac{1}{30}$ that of the fluffy material, depending on conditions of washing, and it has high succinoxidase activity relative to protein-nitrogen. The fluffy material has the high ratio of pentosenucleic acid to protein-nitrogen, considered characteristic of the microsome fraction, but its succinoxidase activity relative to its protein-nitrogen content is also high—about 80 per cent of that of the densely packed material.

On examination with the phase microscope, both mitochondrial sub-fractions are found to be composed of visible particles, which are mainly short rods, although some vesicles of the sort described by Harman (Exper. Cell Research, **1**: 394, 1950) as characteristic of mitochondria are present in both sub-fractions. In the microsome fraction no particles are visible by phase microscopy. On electron microscopy, the particles of the fluffy mitochondrial layer are seen to be about $\frac{1}{2}$ the length and width of those of the densely packed layer.

The two mitochondrial sub-fractions and the microsome fraction as well are stained by dilute solutions of Janus Green B. On incubation at 37° C., however, only the densely packed mitochrondrial layer reduces the dye to safranin, if no substrate is added. On addition of succinate prior to incubation at 37° C., all three fractions reduce Janus Green B.

CERTAIN BIOLOGIC CHARACTERISTICS OF THE RABBIT V_2 CARCINOMA.

EDWIN A. LAWRENCE, DONALD B. MOORE,* and GEORGE I. BERNSTEIN.* (Department of Surgery, Indiana University Medical Center, Indianapolis, Ind.)

During the last 2 years the rabbit V_2 carcinoma, described first by Kidd and Rous (J. Exper. Med., 71: 813, 1940), has been carried through twelve generations of transplants in this laboratory in the course of associated experimental studies. In these investigations, thigh muscles, subcutaneous tissues, and the caval and vertebral venous systems have all been employed at various times as recipient sites. Furthermore, a variety of methods has been used in transplanting the tumor, e.g., by tumor fragments and suspensions prepared by tissue mincers and the Waring Blendor.

Characteristics of the growth of the tumor under the conditions outlined above are described and discussed together, with some comments on features of the tumor that are associated with the regression of growth that occasionally occurs and the apparent immunity that is thereby induced in the host animal.

STUDIES ON THE *IN VITRO* SYNTHESIS OF GLYCINE INTO PURINES AND PROTEINS.

G. A. LEPAGE. (McArdle Memorial Laboratory, University of Wisconsin, Madison, Wis.)

Cell suspensions used in this study include those of the Ehrlich ascites cell carcinoma, the Gardner lymphosarcoma in ascites cell form, and suspensions of mouse liver cells supplied by Dr. J. P. Kaltenbach (Fed. Proc. [in press]). Incorporation of radioactivity from glycine-2-3^{14} into the isolated proteins and the nucleic acid purines as measured after incubation in Robinson's medium (Biochem. J., **45**: 68, 1949) with glucose. Guanine always contained more radioactivity than adenine. Tumor cell proteins were labeled to a several-fold greater extent than those of liver cells.

Experiments with homogenates in fortified media gave approximately $\frac{1}{10}$ the incorporation obtained with whole cells. Tumor ascites cells were difficult to homogenize. Radioactivity was detected in the acid-soluble adenine nucleotides of liver homogenate experiments at a higher level than in the nucleic acid adenine. The large pool of acid-soluble adenine nucleotides may therefore have an influence on the amount of incorporation into nucleic acid adenine and on the relative specific activities of adenine and guanine.

THE ESTIMATION OF NUCLEIC ACIDS IN INDIVIDUAL ISOLATED NUCLEI OF ASCITES TUMORS BY ULTRAVIOLET MICROSPECTROPHOTOMETRY AND ITS COMPARISON WITH THE CHEMICAL ANALYSIS.

CECILIE LEUCHTENBERGER, GEORGE KLEIN,* and EVA KLEIN.* (The Wallenberg Laboratory for Experimental Cytology at the Institute for Cell Research, Karolinska Institutet, Stockholm, Sweden.)

Previous work by C. and R. Leuchtenberger and C. and R. Vendrely on normal tissue has shown that it is possible to determine quantitatively the amount of nucleic acids in isolated individual nuclei by using ultraviolet microspectrophotometry. In the present study the nucleic acid content of the Ehrlich and DBA lymphoma ascites tumor was studied by the same method and compared to the data of the biochemical analyses on the same nuclei.

The microspectrophotometric analyses showed that the DNA in individual nuclei of Ehrlich ascites tumor was approximately twice the amount of the DNA of normal cells, while the DNA of the DBA lymphoma was approximately the same as that of the normal nuclei. The RNA in the nuclei, however, was much higher in both tumors (about 25 per cent of the total NA) than the RNA of nuclei of normal cells (about 5 per cent of total NA). These cytochemical findings are in accordance with the results of previous and present chemical analyses by Klein and Klein.

A CYTOCHEMICAL STUDY OF DNA IN SENILE KERATOSIS. CECILIE LEUCHTENBERGER and HERBERT Z. LUND. (Department of Zoölogy, Columbia University, New York, N.Y.)

Senile keratosis is considered to be a precancerous lesion in the sense that it is a phase of a progressive dermatosis which often results in the development of carcinoma. The dermatosis occurs in individuals who are susceptible to the damaging effects of sunlight, and it is characterized by degeneration of the collagenous layer of the cutis, hyperkeratosis, and, in a well advanced case, atypia of the cells of the epidermis. Hyperplasia may or may not be present. The epidermal cells show alteration of the mechanism of keratinization and variation in nuclear size and staining. We wish to show that in the typical lesion (senile keratoma of Freudenthal) the content of DNA in the nuclei, as estimated by microspectrophotometry, varies indiscriminately from normal to very high values. This is contrasted with the normal skin in which the nuclei have a uniform DNA content and verruca vulgaris in which the content is variable but in distinct multiples.

THE CONCENTRATION OF PNA AND DNA IN DBA MOUSE ASCITES THYMOMA AS A FUNCTION OF AGE AND GROWTH RATE OF THE TUMOR. HILTON B. LEVY,* HAROLD M. DAVIDSON,* and ARTHUR L. SCHADE* (introduced by Dean Burk). (Overly Biochemical Research Foundation, New York, N.Y.)

Earlier work from this laboratory showed that during the life cycle of the bacterium, *P. vulgaris*, and the yeast, *Saccharomyces cerevesiae*, the growth rate and the synthetic activity of the cultures were proportional to the concentration of pentosenucleic acid (PNA), all three metabolic quantities showing a sharp rise in the early stages of growth. We have now investigated the *in vivo* culture of the free cells of the mouse ascites tumor to determine the possible relationship between the synthetic activity of the tumor cells and their PNA concentration.

A group of DBA mice was injected with thymoma cells. At intervals of time up to 13 days after injection, three animals were sacrificed, and the concentration of PNA and DNA of the tumor cells (among other metabolic quantities) were determined. The PNA concentration went up markedly during the early stages of most rapid growth and then declined. The DNA concentration also went up, but only slightly, and then declined. As a consequence, the ratio of PNA/DNA rose to a maximum at the time of maximum growth rate and then declined.

CALCIUM TOLERANCE TEST, AN INDICATOR OF BONE METABOLISM AND OF THE EFFECTS OF THERAPY THEREON. ISAAC LEWIN* and HERTA SPENCER.* (Division of Neoplastic Diseases, Montefiore Hospital, New York, N.Y.)

The need for a simple and rapid test that will help to define the state of bone metabolism in diseases affecting the skeletal system is widely recognized. Such a test should reflect early changes of bone activity and should be a guide in judging the effects of therapy. These requirements appear to be met by measuring the 24-hour urinary calcium excretion following the intravenous infusion of a test-dose of calcium gluconate. Significant differences in the calcium excretion rates were found between "normals" and groups of patients with increased tendency to bone destruction or of bone formation, respectively. The test is sensitive and reflects spontaneous fluctuations of bone metabolism and changes induced by therapy. The effects of ACTH and of male and female sex hormones on neoplastic and metabolic bone diseases will be discussed. The usefulness of this test in exploring the mechanism of derangements of mineral metabolism in hyper- and hypocalcemia of unknown nature will be illustrated with cases of Boeck's sarcoid, Cushing's syndrome, and idiopathic steatorrhea.

The results of these investigations indicate that this test is a sensitive tool which reflects changes of bone metabolism earlier and more accurately than clinical, laboratory, and x-ray studies.

EFFECT OF LA^{139}- AND LA^{140}-CHLORIDE ON MICE BEARING EHRLICH ASCITES TUMORS. RUTH LEWIN,* HERTA SPENCER,* DANIEL M. EKSTEIN,* LEONARD

WOIDOWSKY,* KURT G. STERN, and DANIEL LASZLO. (Division of Neoplastic Diseases, Montefiore Hospital, New York, N.Y., and The Departments of Chemistry and Physics, Polytechnic Institute of Brooklyn, New York, N.Y.)

Investigations concerned with the distribution of rare earth elements in normal mice have shown that intraperitoneally injected $LaCl_3$ remained localized mainly within the peritoneal cavity. Therefore, it appeared of interest to study the effect of stable and radiolanthanum chloride on mice bearing Ehrlich ascites tumor. More than 500 mice (Strains A, C3H, and CFW) were inoculated with 10^4 to 10^7 ascites tumor cells. Three to 5 days after inoculation, groups of 10–30 animals were injected intraperitoneally with (*a*) La^{140}-chloride (100–400 μc.) containing stable carrier La^{139}-(0.5–2.0 mg.), (*b*) La^{139}-chloride in amounts equivalent to the carrier contained in the radioactive solution, and (*c*) saline. The rate of formation of ascites fluid and the multiplication of tumor cells were markedly inhibited by radiolanthanum, whereas stable lanthanum was much less effective. The survival time of La^{140}-treated animals was considerably prolonged. These effects of radiolanthanum appear to be related to its high concentration in the ascites sediment which contains the tumor cells. The results of lanthanum determinations on the tumor cells (separated by the albumin flotation method), autoradiography, and histological and cytochemical studies will be presented.

ISOLATION AND PROPERTIES OF RAT LIVER CELL NUCLEOLI.

MORTIMER LITT,* KENNETH J. MONTY,* and ALEXANDER L. DOUNCE. (Department of Biochemistry, The University of Rochester School of Medicine and Dentistry, Rochester, N.Y.)

Nucleoli have been isolated from previously isolated nuclei of rat liver in a reasonably pure state. The nuclei are suspended in 1 per cent gum arabic at pH 6.2 to 6.3 and are then disrupted by the use of a sonic oscillator. Following one sedimentation by gravity to remove large particles, the nucleoli are recovered by centrifugation and are then resuspended in distilled water. Further purification is achieved by differential centrifugations in distilled water and 2 per cent gum arabic solution at pH 6.0. The nuclei used in this work are isolated in 1 per cent gum arabic solutions at pH 6.0. All operations are carried out near 0° C.

By direct analysis, the nucleoli appear to contain about 17–18 per cent desoxyribonucleic acid and only a few per cent ribonucleic acid. The Feulgen staining reaction is positive on all isolated nucleoli examined in smears, although the intensity of the coloration varies. The nucleoli give the usual protein tests, including a positive Millon's reaction, and give a positive test (qualitative) for catalase. In regard to morphology, many of the nucleoli when freshly isolated can be seen, by observation with the oil immersion objective, to contain a number of very tiny, apparently elongated bodies. Most of the nucleoli have been broken off from the chromosomes to which they presumably were originally attached, but a small percentage are still attached to chromosomes.

TECHNICAL PROBLEMS IN TUMOR TRANSPLANTATION.

JOHN B. LOEFER. (Foundation of Applied Research, San Antonio, Texas.)

The tumor used was a transplantable fibrosarcoma which has been propagated in Sherman strain rats for 4 years. The incidence of successful grafts has been demonstrated to differ significantly (*a*) if the transplantation technic is altered, (*b*) when the tumor itself varies, e.g., its age or viability, as affected by the animal bearing the stock, and (*c*) when the condition of the recipient is modified.

Subcutaneous transfer with a trocar is the preferred method. Transplants with finely macerated or homogenized tissue have been unsuccessful. Implants of 45 mg. produced a significantly greater number of takes than those averaging 10 mg. Whether tissue is taken from the periphery or interior of a stock tumor did not alter takes significantly. Maintaining the tumor fragments at 37° in the $\frac{1}{2}$- to 1-hour interval which occurs while making a series of transplants causes a decrease in successful implants as compared to results obtained at 22°–24° C.

The age of the tumor or of the animal bearing the stock may have a marked effect on the number of successful grafts. The age of the graft recipients and their dietary histories are, likewise, important determining factors.

It is emphasized that it is essential to control all known variables in experiments which utilize successful graft incidence as a criterion of effect, and, since there is considerable variation among individual rats, sufficient animals must be used so that results can be statistically evaluated. If so used, the method is a reliable experimental tool.

THE TUMOR-DEPRESSANT EFFECT OF THE NONDIALYZABLE BUFFER-SOLUBLE FRACTION OF PODOPHYLLIN.

M. R. Loran* and A. Towbin.* (College of Pharmacy and the Department of Pathology; Ohio State University, Columbus, Ohio.)

The nondialyzable buffer-soluble fraction recently isolated from podophyllin has been shown to possess biologic activity when administered to experimental animals. This fraction was separated from podophyllin by a process of dialysis and centrifugation and differs from other reported derivatives in its physical-chemical properties.

In the present investigation the effect of the fraction on transplanted Sarcoma 37 was studied. The oncolytic capacity of the agent was demonstrated by injecting a single massive dose intraperitoneally; the maximal effect was manifested on the fifth day; the treated tumors averaged 11 per cent that of the controls at this point. Grossly, the tumors became brown and indurated; microscopically, they were composed largely of necrotic debris. In subsequent days beginning regrowth of the tumors was evident.

Efforts to effect stasis of tumor growth by small repeated doses were not successful; in the latter part of this experiment the accumulative action of the agent effected a lytic response in the tumors. The effect of the agent on tumor transplantability was studied by injecting divided doses beginning directly after transplantation. A slight retardation in time of appearance of the tumors in the treated animals was evident.

With the dosage pattern used in these experiments severe toxic systemic reactions occurred.

STUDIES ON THE METABOLISM OF VARIOUS N-METHYL-C^{14}-SUBSTITUTED AMINOAZO DYES.

J. C. MacDonald,* A. M. Plescia,* E. C. Miller, and J. A. Miller. (McArdle Memorial Laboratory, University of Wisconsin, Madison, Wis.)

Miller, Plescia, Miller, and Heidelberger (Cancer Research, **11:** 268, 1951) showed that a small but significant amount of the C^{14} administered as 3′-methyl-4-dimethyl-C^{14}-aminoazobenzene to adult male rats was incorporated into the methyl groups of choline and the β-carbon of serine. On the other hand, Boissonnas, Turner, and du Vigneaud (J. Biol. Chem., **180:** 1053, 1949) found no radioactivity in the choline methyl groups of an immature female rat given 4-dimethyl-C^{14}-aminoazobenzene.

In a comparison of the metabolism of these strong carcinogens to that of two weak carcinogens, 4′-methyl-4-dimethyl-C^{14}-aminoazobenzene and 3-methyl-4-monomethyl-C^{14}-aminoazobenzene, in adult male rats the C^{14} from each dye was incorporated into the choline methyls and the β-carbon of serine to approximately the same extent. Immature or adult female rats also incorporated C^{14} from 4-dimethyl-C^{14}-aminoazobenzene to a similar degree. The above compounds, 4-monomethyl-C^{14}-aminoazobenzene, and its 3′-methyl and 4′-methyl derivatives were metabolized to $C^{14}O_2$ at approximately the same rate; this rate was not influenced by prefeeding nonradioactive dye for 3 weeks prior to the test dose. Thus, no difference was found in the metabolism of the N-methyl groups from strong or weak carcinogens.

The fate of the N-methyl groups apparently involves an oxidation to N-hydroxymethyl groups (see also Mueller and Miller, Cancer Research, **11:** 271, 1951). The major fraction of this derivative probably breaks down to formaldehyde which is used for the synthesis of labile methyl groups or the β-carbon of serine or is oxidized to CO_2. A small fraction of the hydroxymethyl derivative is presumably involved in the formation of protein-bound dye.

EFFECT OF CARCINOGENS AND CANCER CHEMOTHERAPEUTIC AGENTS UPON ANTIBODY FORMATION IN MICE.

Richard A. Malmgren* and Bertrand E. Bennison* (introduced by Howard B. Andervont). (National Cancer Institute, Bethesda, Md.)

The antibody-depressing effect of the carcinogens, nitrogen mustard, x-ray, and the Rous sarcoma virus, and the chemotherapeutic agents cortisone and benzene is well established. The present studies were undertaken to determine whether or not this antibody inhibition is a property common to other substances having carcinogenic or chemotherapeutic effects.

The compounds were tested in strains C (B alb C) and C3H mice. The carcinogens were given subcutaneously 3 times in a 12-day period, and the chemotherapeutic agents were injected subcutaneously one a day for 5 days. A 4 per cent suspension of sheep red cells, used as antigen, was injected intraperitoneally 5 days before the sera were harvested.

The carcinogens, urethan, methylcholanthrene, 1,2,5,6-dibenzanthracene, 1,2-benzanthracene, and 4-dimethylaminoazobenzene all depressed the hemolysin titers, while the noncarcinogenic analogs of these compounds (methyl carbamate, phenanthrene, and 4-diethylaminoazobenzene) did not affect the formation of antibodies.

The chemotherapeutic agents, alpha peltatin, A-methopterin, benzene, colchicine, 2-6-diamino

purine, podophyllotoxin, sodium arsenite, and triethylmelanine all depressed antibody formation. One compound, furacin, did not lower the antibody titer.

Since passive antibodies *in vivo* and *in vitro* were not affected by any of these compounds, it is inferred that they act by interfering with normal antibody production.

The possibility that the lowered hemolysin titer was a result of general systemic toxicity produced by these compounds was considered and subsequently ruled out. Maximum tolerated doses of NaCN and $CuSO_4$ did not decrease antibody production. Further, the administration of a carcinogen and its noncarcinogenic analog in amounts of equal toxicity resulted in inhibition by the carcinogen only.

Antibody inhibition at the site of antibody production appears to be a property common to these carcinogenic and cancer chemotherapeutic substances.

BEHAVIOR OF I^{131} ANTI-MAMMARY TUMOR MICROSOME FRACTION IN MICE. S. P. Masouredis,* L. R. Melcher,* and M. B. Shimkin. (Laboratory of Experimental Oncology, National Cancer Institute, and the Division of Medicine, University of California School of Medicine, San Francisco, Calif.)

The behavior of I^{131}-labeled rabbit anti-ovalbumin (ANTI-OV) and of anti-mouse mammary tumor microsome (ANTI-MTM) was studied in C3H and C mice following intravenous administration. The microsome antigen, which was biologically active for milk agent, was prepared by differential centrifugation of a mammary tumor homogenate obtained from C3H mice.

The rate of excretion of radioactivity in C mice receiving ANTI-OV was exponential, with half being excreted in 110 hours. Two components were involved in the excretion of mice receiving ANTI-MTM, an initial rapid rate with a half-time of less than 10 hours, and a slow component identical with that found with ANTI-OV. The concentration-time course of radioactivity in the plasma as well as the tissue radioactivity suggested that 15–20 per cent of the ANTI-MTM leaves the plasma at a rate 10 times as fast as the ANTI-OV. This component was localized transiently in some tissue pool which included the liver, kidney, and spleen, and the I^{131} associated with this component was then excreted.

Despite the marked difference in metabolic fate of ANTI-OV and ANTI-MTM in mice, no significant difference in the behavior of ANTI-MTM was found between mice with the milk agent and mice free of the agent. This suggests that the antitissue components in the ANTI-MTM overwhelm and probably mask the behavior of the anti-milk agent.

THE CELLULAR LOCALIZATION OF MATERIAL AFTER THE ADMINISTRATION OF THE CARCINOGEN, β-NAPHTHYLAMINE: A STUDY BY FLUORESCENCE MICROSPECTROSCOPY. Robert C. Mellors. (Sloan-Kettering Institute for Cancer Research and Department of Pathology, Memorial Center, New York, N.Y.)

This contribution describes the use of fluorescence microspectroscopy of individual cells in a search for intracellular material that is related to or derived from an administered carcinogenic, fluorescent compound, β-naphthylamine (I).

After the oral ingestion of (I) by the female dog, a material with a fluorescence spectrum having a maximum at 430–440 mμ and differing from that of the ingested carcinogen occurs in the urine, in the exfoliated epithelial cells of the urinary tract, and in the superficial and deep transitional cells of the biopsied bladder mucosa (R. C. Mellors and J. Hlinka, Cancer [in press]). The material is not found in exfoliated epithelial cells of the gastric mucosa despite the oral intake of (I).

The characteristic fluorescence occurs in the epithelial cells of the bladder of a dog having ureteral transplants by which the urine flow is directed into the large bowel rather than into the bladder. Therefore, the bladder mucosa can acquire the fluorescing material both by resorption from the urine and by way of the circulating blood.

NUCLEIC ACID CONTENT PER CELL OF BONE MARROW ASPIRATED FROM PATIENTS WITH LEUKEMIA. Maud L. Menten. (British Columbia Medical Research Institute, Vancouver General Hospital, Vancouver, Canada.)

Nucleic acid was determined by the method of Schmidt and Thannhauser (J. Biol. Chem., **161:** 83, 1945) on 1–2 ml. of 10 per cent aqueous suspension of bone marrow aspirated from patients with leukemia. Amount of nucleic acid per cell was calculated from the number of cells enumerated for 1 c.mm. of marrow.

Both myelogenous and lymphatic leukemias showed values ranging from 0.42×10^{-9} mg. to 4.5×10^{-9} mg. per cell. Lymphocytes from a surgically removed spleen reached 6.5×10^{-9} mg. per

cell. Minimal amounts were found in leukemia with the most anaplastic type cell; higher values occurred with increasingly differentiated cell types. This relationship was lost following roentgen radiation, which considerably reduced cellular nucleic acid. The values obtained did not form a continuous unbroken succession but displayed periodicity with separation into groups. Each succeeding group was augmented by roughly 50 per cent of the mean amount occurring in the preceding one. Groups were less well defined in the series of myelogenous and lymphatic leukemias than in the series obtained in developing red blood cells, where a characteristic type of immature nucleated cell preponderated in different dysplasic arrests of maturation. Results of this latter study are being submitted for publication.

The variation in amounts of cellular nucleic acid observed in leukemias did not conform to the theory of constancy of value of desoxyribonucleic acid postulated for nuclei of diploid somatic cells by Boivin, Vendrely, and Vendrely (Compt. rend. Acad. d. sc., **226**:1061, 1948).

THE ROLE OF VASCULARIZATION IN THE SURVIVAL OF SUBCUTANEOUS HOMOGRAFTS IN MICE.

Ruth M. Merwin and Elizabeth L. Hill.* (National Cancer Institute, Bethesda, Md.)

Transplants made among mice of different inbred strains were observed *in vivo* in a transparent chamber in the skin of adult mice. The tissues transplanted were thyroid and Harderian gland from late embryos or mice a few days old. Vascularized implants were always destroyed. When nonvascularized implants only were present, they survived indefinitely, but in the presence of vascularized tissue they were destroyed. Even after 9 months' survival in a foreign strain a nonvascularized implant was destroyed when a new implant in the same host was vascularized. Thus, under the present experimental conditions vascularization is necessary for the initiation of resistance, but vascularization is not necessary for vulnerability to the destructive processes attendant on the resistant state.

Implants vascularized within a week after transplantation disintegrated, on the average, 10 days later, while those vascularized at 2 weeks disintegrated 22 or more days later. Partial thyroidectomy increased the period of survival of thyroid implants.

A STUDY OF THE FACTORS INVOLVED IN THE INHIBITION PRODUCED BY LARGE DOSES OF ESTROGEN ON TRANSPLANTABLE MAMMARY FIBROADENOMA IN RATS.

M. Jean Millar* and R. L. Noble. (Department of Medical Research, University of Western Ontario, London, Canada.)

The effects of various hormone preparations on the growth of a transplantable benign fibroadenoma in rats have been studied.

Stimulation of tumor growth has been shown, with pregnancy, with the administration of small doses of estrogen, and with the administration of crude anterior pituitary preparations. Inhibition of tumor growth occurs with ovariectomy and with the administration of large doses of estrogen (diethylstilbestrol) in males and females.

The possibility of the tumor inhibitory effect of large doses of stilbestrol being a nonspecific result of the general body weight depression has been investigated. Animals maintained, by dietary restriction, at the same weight level as stilbestrol-treated rats did not produce the same tumor inhibition as seen with the hormone treatment.

In view of the stimulatory effect of anterior pituitary preparations, the possibility that the large doses of stilbestrol act by depressing growth hormone output is considered.

ON THE INHIBITORY ACTION OF CERTAIN POLYCYCLIC HYDROCARBONS ON AZO DYE CARCINOGENESIS.

E. C. Miller, J. A. Miller, and R. R. Brown.* (McArdle Memorial Laboratory, University of Wisconsin, Madison, Wis.)

Richardson and Cunningham (Cancer Research, **11**: 274, 1951) observed that rats treated simultaneously with 3′-methyl-4-dimethylaminoazobenzene (3′-Me-DAB) and 20-methylcholanthrene (MC) developed liver tumors much more slowly than rats fed 3′-Me-DAB alone. In extending these studies we found that the addition of 0.12 mM per kilo of MC, 3,4-benzpyrene, 1,2,5,6-dibenzanthracene, or 1,2-benzanthracene (BA) to a diet containing 0.054 per cent (2.4 mM per kilo) of 3′-Me-DAB inhibited tumor induction; pyrene (PY) was without effect. Tumors were not found in rats fed 0.027 per cent of 3′-Me-DAB.

The levels of 3′-methyl-4-aminoazobenzene in the blood and of free and protein-bound dyes in the livers of rats fed 3′-Me-DAB with an inhibitory hydrocarbon were lower than in rats fed 0.054 per cent of dye alone or with PY, and similar to those in rats fed only 0.027 per cent of 3′-Me-DAB. The ability of liver homogenates from rats fed 0.054 per cent of 3′-Me-DAB alone or with PY to N-demethylate and reduce azo dyes were

markedly lowered. These enzymatic activities were maintained at higher levels in rats fed 0.027 per cent 3′-Me-DAB or 0.054 per cent of 3′-Me-DAB plus either MC or BA.

These data suggest that the hydrocarbons inhibit azo dye carcinogenesis by maintaining the ability of the liver to metabolize the dyes, thereby reducing the effective dose. These data are of further interest, since the feeding of various peroxides or polycyclic hydrocarbons causes a marked increase in the capacity of mouse livers to metabolize azo dyes (Brown, Miller, and Miller, Fed. Proc., 1952).

AN ELECTROPHORETIC STUDY ON THE ORIGIN OF THE ABNORMAL PLASMA PROTEINS IN MULTIPLE MYELOMA.

Gail Lorenz Miller,* Clark E. Brown,* Elizabeth Eshelman Miller,* and Edward S. Eitelman* (introduced by Mary Adelia Bennett). (The Institute for Cancer Research, the Lankenau Hospital Research Institute, and the Lankenau Hospital, Philadelphia, Pa.)

An electrophoretic study was made of the plasma and extracts of tumor of a typical multiple myeloma subject. Tumor was obtained at autopsy from the vertebrae, ileum, and lymph nodes. Control tests were made with corresponding normal plasma and normal tissues, and with tumor from a myelogenous leukemia subject. Vertebral marrow obtained from a multiple myeloma subject that had undergone urethan treatment also was investigated. A large, sharp peak, having a mobility somewhat lower than that of normal gamma globulin, characterized the electrophoretic pattern of the plasma and the tumor extracts of the test subject. In special studies, an effort was made to exclude the effects of blood or lymph as possible contaminants in the tumor extracts. The characteristic sharp peak was not observed in any of the control experiments. It was also absent in the extract of the marrow of the urethan-treated multiple myeloma subject, due, presumably, to the effect of the urethan in inhibiting the growth of the myeloma cells. The results appear to support the view that myeloma cells are the site of formation of the abnormal plasma proteins of multiple myeloma.

ON THE CARCINOGENICITY AND METHYLATION *IN VIVO* OF 4-AMINOAZOBENZENE AND ITS 3′-METHYL AND 4′-FLUORO DERIVATIVES IN THE RAT.

J. A. Miller and E. C. Miller. (McArdle Memorial Laboratory, University of Wisconsin, Madison, Wis.)

The hepatocarcinogens related to 4-dimethylaminoazobenzene (DAB) require at least one N-methyl group for strong activity in the rat. One of the principal facts supporting this has been the failure of many workers to induce tumors with 4-aminoazobenzene (AB). However, Kirby (Biochem. J., **39**: xxxiii, 1945; Cancer Research, **7**: 333–41, 1947) succeeded in demonstrating under very drastic conditions that AB has a carcinogenic activity of a very low order in the rat. He concluded that ". . . methylation of the primary amine, 4-aminoazobenzene, enhances but does not initiate carcinogenic activity. . . ."

We have now found that 3′-methyl-AB has a low but easily demonstrable carcinogenic activity. 4′-Fluoro-AB was only slightly active, and AB was again inactive under our conditions. The 3′-methyl and 4′-fluoro substitutions are known to approximately double the activity of DAB. It has also been possible with improved methods to show that these primary aminoazo dyes are methylated, chiefly to the N-monomethyl derivative, to a small extent *in vivo*. The 3′-methyl derivative is methylated to a greater extent than is either of the other dyes. 3′-Methyl-AB also formed much larger amounts of protein-bound dye than did the other dyes. Thus, these primary aminoazo dyes appear to be carcinogenic to the extent that they are methylated *in vivo* and bound to the liver protein. The latter reaction requires an N-methyl group.

FURTHER STUDIES ON THE CARCINOGENIC ACTIVITIES OF DERIVATIVES OF 2-ACETYLAMINOFLUORENE IN THE RAT.

J. A. Miller, E. C. Miller, R. B. Sandin,* and H. P. Rusch. (McArdle Memorial Laboratory, University of Wisconsin, Madison, Wis., and the Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada.)

Under our conditions 2-acetylaminofluorene induces liver tumors in male rats, mammary tumors in female rats, and tumors of the ear duct and small intestinal epithelium in both sexes. Alterations in structure were found to affect the activity toward a given tissue and the tissue specificity.

Liver tumors were induced only by compounds with the $-CH_2-$ bridge (2-acetylamino-, 7-fluoro-2-acetylamino-, 2-dimethylamino-, 2-amino-, and 2-nitrofluorene) or by compounds (2-acetylamino-

fluorenone and 2-acetylaminofluorenol) which could theoretically be reduced *in vivo* to yield this structure. Fluorine in the 7-position greatly increased the activity of 2-acetylaminofluorene as a hepatic carcinogen in both male and female rats.

The structural requirements for the induction of mammary tumors are less specific than for the induction of tumors at the other sites. 4-Acetylaminobiphenyl was as active a mammary carcinogen as 2-acetylaminofluorene. The introduction of a methyl group in either the 2- or 2′-positions of 4-acetylaminobiphenyl (with a resultant loss of planar structure) abolished the carcinogenic activity of the compound. Compounds with bridges (-S-, -O-, -SO-, -CH=CH-, $-CH_2-CH_2-$) between these positions were active; presumably these molecules are planar. No compound which failed to induce mammary tumors in female rats produced tumors at other sites in rats of either sex.

Tumors of the ear duct and small intestinal epithelium were induced by most, but not all, of the compounds which were active as mammary carcinogens. The unbridged compounds were generally less active at these sites than for the mammary tissue.

2-Acetylaminophenanthrene also induced myelogenous and lymphatic leukemia.

EFFECT OF VITAMIN C-FREE DIET ON RADIOSENSITIVITY OF MALIGNANT TUMORS.

Theodore L. Miller,* Boris Sokoloff, and Walter H. Eddy. (Pack Medical Group, N.Y., and Southern Bio-Research Lab., Florida Southern College, Lakeland, Fla.)

An almost complete withdrawal of ascorbic acid from the system increases the radiosensitivity of Crocker rat carcinoma; 3,000 or 5,000 r contact radiation applied to 18 c.mm.-size carcinoma of rats on regular diet produces a partial destruction of the tumor. Sixty rats were placed on a Sherman-LaMer diet to which 5 per cent of D-gluco-ascorbic acid and 0.5 per cent of flavonoids were added. When the size of tumor reached 19–20 c.mm., 30 rats were submitted to 3,000 r contact radiation in a single dose and 30 rats to 5,000 r. All tumors were completely destroyed, and all rats except one survived. Radiation factors were the same in all experiments. Ascorbic acid content of plasma was 0.025 mg/100 cc of plasma (average). These experiments indicate that a temporary but almost complete withdrawal of ascorbic acid from the system results in the same complete destruction of Crocker carcinoma with smaller doses of radiation (3,000 r and 5,000 r), as is achieved with much larger doses (8,000 r and 10,000 r) when the ascorbic acid level is normal. Nine patients with various types of malignant growth were placed on a Vitamin C-free diet. After their ascorbic acid content was brought down, radiation therapy was applied.

USEFULNESS OF SERUM CHOLINESTERASE DETERMINATION IN LIVER DISEASE AND MALIGNANCY: A PRELIMINARY REPORT.

David W. Molander,* Max M. Friedman,* and John S. LaDue.* (Pack Medical Group, New York 16, N.Y.)

Serum cholinesterase activity has been determined in 120 normal controls and in 100 other patients by a colorimetric method that compares favorably with Michel's potentiometric method. In addition, several other standard liver function tests have been determined.

Low levels have been found in patients with acute parenchymal liver disease. The levels have varied in patients with cirrhosis. In patients with widespread metastatic carcinoma with metastases to the liver, low levels have generally been found. In cases of lymphoma with liver involvement, low levels have been consistently seen. In general, in patients that have more extensive disease, lower levels have been observed than in those with less involvement. In patients with lymphoma, serum cholinesterase levels have progressed toward normal with treatment and clinical improvement.

In some cases where the other liver function tests have not been greatly altered, the serum cholinesterase has been low and has been a useful guide to the clinician in planning the management of these patients. Serum cholinesterase activity has been helpful in interpreting the values of other liver function tests, particularly in patients with parenchymal liver disease and intrahepatic biliary obstruction where elevated alkaline phosphatase levels may falsely lead to surgical intervention.

The distinct clinical impression has been gained that serum cholinesterase activity is a further useful liver function test in detecting liver disease, in following improvement in cases of lymphoma with liver involvement, in evaluating patients with possible liver metastases, and, finally, in differentiating the etiology of jaundice in various patients.

EFFECT OF CORTISONE AND SEX STEROIDS ON THE INDUCTION AND MAINTENANCE OF CASTRATION-INDUCED ADRENAL CORTICAL ADENOMAS OF MICE.

Harry Monsen* (introduced by Arthur Kirschbaum). (Department of Anat-

omy, College of Medicine, University of Illinois, Chicago, Ill.)

A study of the influence exerted by anterior pituitary hormones on the development of castration-induced adrenal cortical adenomas in NH mice was made by administering sex steroids and cortisone to ovariectomized animals.

Weekly doses of 250 μg. testosterone propionate prevented the occurrence of adenomas if treatment was begun at the time of ovariectomy but did not alter the histological picture of established adenomas.

Daily administration of 400 μg. cortisone caused a decided atrophy of the adrenal gland, affecting primarily the zona fasciculata, but did not inhibit adenomatous growth originating in the subcapsular region. No retrogressive change resulted in established adenomas from cortisone treatment.

If a castration-induced elevated blood level of circulating gonadotrophins acts upon the adrenal cortex to produce the pathologic changes, the ineffectiveness of testosterone in treating the established tumor may be explained on the basis of pituitary refractoriness to exogenous androgens at this stage or of the autonomy of the adenoma.

Cortisone apparently depressed the elaboration of ACTH sufficiently to cause atrophy of the adrenal cortex but did not prevent the induction of adenomas.

Although complete suppression of pituitary ACTH production may not have been obtained with the employed dose of cortisone, it is reasonable to conclude that ACTH is not directly responsible for the genesis of these adrenal cortical adenomas.

HISTOGENESIS OF GASTRIC CARCINOMA.

R. M. Mulligan and R. R. Rember.* (Department of Pathology, University of Colorado School of Medicine, Denver, Colo.)

Of 62 patients with gastric carcinoma, 54 were males and eight were females; they were 32–91 years old; 59 had one gastric carcinoma and three had two. These 65 carcinomas were divided into five main types, as follows: mucous-cell, 45; intestinal-cell, nine; pancreatic heterotopia, four; chief-cell, four; and pylorocardiac gland-cell, three. A carcinoma of any type confined to the mucosa was classified as Stage I; invaded into muscle coats, Stage II; invaded to serosa, Stage III; and invaded into adjacent viscera and/or metastasized, Stage IV. The mucous-cell type was thought to be derived from cells lining the surface and mouths of the glands throughout the gastric mucosa; the intestinal-cell type, from glands of intestinal type, heterotopic or metaplastic, in gastric mucosa; the pancreatic heterotopia type, from pancreatic duct cells heterotopic in gastric submucosa; the chief-cell type, from chief cells of the fundic mucosa of the stomach; and the pylorocardiac gland type, from specialized pyloric and cardiac glands. On the basis of location, size, stage, culpability in causing death, histologic characteristics, ability to directly invade extragastric tissues, and spread and extent of metastases, the following descending order of malignancy was found for these five types of gastric carcinoma: mucous-cell, intestinal-cell, pancreatic heterotopia, chief-cell, and pylorocardiac gland-cell.

RADIOACTIVE Au^{198} IN GOLD SEEDS FOR CANCER THERAPY.

William G. Myers and Benjamin H. Colmery, Jr.* (Division of Biophysics and Radioisotopes of the Medical Center, and the Department of Physics, The Ohio State University, Columbus 10, Ohio.)

Au^{198}, half-life 2.70 days, was generated in pure gold wire, 0.007 inches in diameter, in the pile at Oak Ridge. The radioactive wire was slid into gold tubing, 0.015 inches inside by 0.032 inches outside in diameter, placed within a protective lead shield. Blunt pliers were used to pinch off uniform seeds, adjusted in length to obtain constant source strengths at various times. The tubing absorbed all the undesirable beta particles (0.970 Mev max.) except for an insignificant leakage at the thinned parts at the ends. Almost 90 per cent of the 0.411 Mev gamma rays passed through the walls of the seeds.

The seeds were implanted into, or adjacent to, C3HBA adenocarcinoma, 15091a spindle-cell mammary carcinoma, Sarcoma 37, and a localized lymphosarcoma, which had been transplanted into C3H, ABC, CFW, and A mice, respectively. Calipers were used to follow the regressions in the sizes of the tumors during the irradiations. Any residual tissue at the tumor site following the radiation exposure was examined histologically, and bits of it were transplanted into new mice to determine viability.

The cancerocidal doses were found to be:

Lymphosarcoma,	800 ± 200 r
Sarcoma 37,	2600 ± 500 r
C3HBA adenocarcinoma,	3600 ± 700 r
15091a carcinoma,	4200 ± 800 r

The sources of radioactive gold wire in the nonradioactive gold tubing beta-absorber may be made any length, and, because they are easily bent, they are very useful for making gamma-ray

plaques. They may be incorporated in flexible plastic tubing to make radioactive sutures and may be inserted in stainless tubing to make rigid needles.

Because the 0.411 Mev gamma rays from Au^{198} have a half-thickness in soft tissues only about 60 per cent as great as in the case of those from Co^{60} and, since they are also much more readily absorbed than most of the gamma rays emitted by radium, it is felt that Au^{198} may prove to be an advantageous successor to radon for permanent seed implantations, as well as to Co^{60} and to radium for removable sources in interstitial therapy.

SPONTANEOUS NEOPLASMS IN FISHES. VI. THYROID TUMORS IN MARINE FISHES. Ross F. NIGRELLI. (New York Aquarium, New York Zoölogical Society, New York, N.Y.)

Hyperplastic and neoplastic thyroids in marine fishes are uncommon and are of special interest, because these animals live in an environment rich in iodine. The present report deals with such growths in one blue angelfish (*Angelichthys isabelita*), ten sheepshead minnows (*Cyprinodon variegatus*), and four common killifish (*Fundulus heteroclitus*). The tumor in the angelfish was typically adenomatous. It consisted of many small, poorly formed acini, in which the colloid appeared abnormal and showed a diminishing staining reaction to both acid and basic dyes. There was a conspicuous increase in the connective tissue stroma. The growths in the sheepshead minnows and the killifish had the appearance of interacinar adenomas. Histologically, they were characterized by the presence of some empty and numerous acidophilic and basophilic colloid-filled follicles, together with an extensive development of interfollicular acini with and without lumina. Clusters of round acidophilic epithelial-like elements were also present in some regions. In all cases, the thyroid growths were highly destructive, invading the gills and the cartilage, bone, and muscle of the hyomandibular and branchial complexes. There was some infiltration into the walls of the larger blood vessels, but no metastases were found. In the common killifish, dysphagia from compression of the esophagus occurred, and there were considerable pressure effects on the heart, ventral aorta, and afferent vessels. The physical and chemical characteristics of the water in relation to the growths in the killifishes will be discussed.

STUDIES ON NEOPLASMS IN FISHES. VII. SPERMATOCYTOMA IN AN AFRICAN LUNGFISH (*PROTOPTERUS ANNECTENS*). Ross F. NIGRELLI and SOPHIE JAKOWSKA. (New York Aquarium, New York Zoölogical Society, and College of Mount St. Vincent, New York, N.Y.)

A large tumor, identified as a spermatocytoma, occurred in a fish (total length 55.8 cm.) which had been in captivity for approximately 5 years. The growth was a bilaterally symmetrical mass, measuring $50 \times 40 \times 20$ mm., and involved the regions of the posterior part of the urogenital system, large intestine, and cloaca. The growth and adjacent structure have been studied and compared to normal specimens and to the anatomical description of Kerr (1901) and the cytological findings of Wickbom (1945).

The testis in normal Protopterus has previously been described as a complicated network composed of two distinct regions, a long anterior formative part and a comparatively short vesicular portion from which vasa efferentia communicate with several of the posterior kidney tubules. The tumor probably arose in the posterior region of the formative testis. It consisted almost exclusively of cells which appeared to have retained the cytological features of normal spermatogonia and spermatocytes in the first meiotic division. Numerous cells in Metaphase I exhibited typical bivalents; in polar view a characteristic hollow spindle was observed. Degenerated cells were rare, but the network pattern, characteristic of the normal testis, was lost and the number of interstitial cells reduced. The tumor was surrounded by a thick connective tissue capsule containing scattered melanophores and was poorly vascularized, as compared to the normal testicular tissue.

COMPETITIVE ACTION OF 2-THIOURACIL AND URACIL IN AAF-INDUCED CANCER OF THE LIVER. KARL E. PASCHKIS, ROBERT J. RUTMAN,* and A. CANTAROW. (Jefferson Medical College, Philadelphia, Pa.)

The incidence of liver cancer induced in rats by 2-acetaminofluorene (AAF) is decreased by simultaneous administration of 2-thiouracil. This observation raised the question as to whether this effect of thiouracil might be due to competition with uracil in animals receiving AAF.

Four groups of male rats (Wistar descendants), receiving AAF in their diet, were treated as follows: (*a*) no additional treatment; (*b*) one 214-mg. pellet of thiouracil subcutaneously biweekly; (*c*) 250 mg. uracil by stomach tube once daily, 6 times weekly; (*d*) both uracil and thiouracil, as in (*b*) and (*c*). These regimens were continued for 90

days, and animals were sacrificed and examined after 310–415 days.

It was found that uracil overcame the protection afforded by thiouracil against the hepatic lesions induced by AAF, but had no effect upon the thyroid hyperplasia induced by thiouracil. It is believed that uracil is not utilized by normal mammalian cells. This observation suggests that uracil may be utilized by and be a requirement for liver cells exposed to the carcinogenic action of AAF and that thiouracil may act as an antimetabolite under these circumstances.

In order to test this hypothesis further, normal and AAF-treated rats were given uracil-2-C^{14} by subcutaneous and intraperitoneal injection. The animals were sacrificed after 12 hours, and radioactivity measurements were made on isolated nuclear, mitochondrial, microsomal, and supernatant fractions of the liver cells. The results of these studies will be discussed.

HETEROLOGOUS TUMOR GROWTH IN THE CHEEK POUCH OF THE HAMSTER. Donald I. Patt,* Alfred H. Handler,* and Brenton R. Lutz* (introduced by Shields Warren). (Department of Biology, Boston University, Boston, Mass.)

Neoplasms, including both carcinomatous and sarcomatous types, from the frog, guinea pig, mouse, rat, and man have been successfully grown in the membranous cheek pouch of the hamster. With serial transplantation, through several passages (three to seven), a progressive reduction in host reaction with each passage, an increase in persistence, and an increase in the size of the tumor before regression or necrosis have occurred. Histological sections show that malignant tumor cells change little in the cheek pouch, although there are varying degrees of dedifferentiation of some of the more highly differentiated carcinomatous types. The stromal tissue becomes either reduced in amount with each passage, or basically modified so as to resemble more closely the connective tissue of the host. Heterologous transplants have been maintained for considerable time in the hamster host (human fibroliposarcoma, 150 days; rat fibrosarcoma, 365 days; frog renal adenocarcinoma, 419 days). To date, mouse Sarcoma 180 and human fibroliposarcoma have shown metastases from the cheek pouch to the lung and liver. The percentage of "takes" has been nearly 100, with an increase in volume up to 1,000 times (fibrosarcoma of the rat). The neoplasms used were: spontaneous renal adenoma of the frog, benzpyrene-induced sarcoma of the guinea pig, spontaneous mammary adenocarcinoma of the mouse, Sarcoma 180, benzpyrene-induced fibrosarcoma of the rat, and four human neoplasms, i.e., fibroliposarcoma, adenocarcinoma of the caecum, ovarian carcinoma, and scirrhous carcinoma of the breast.

DIFFERENCES IN ALKALINE PHOSPHATASE ACTIVITY AND DISTRIBUTION AMONG SEVERAL TRANSPLANTABLE MAMMARY TUMORS IN MICE. Bjarne Pearson and Flavia Richardson.* (Department of Pathology and Oncology, University of Vermont, College of Medicine, Burlington, Vt., and the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine.)

Four adenocarcinomas of the breast were transplanted into six series of mice with an average of 45 mice to each series. Tumor E 0771 was transplanted into C57 blacks, DbrB into DBA, 15091a into BAF_1, H 2712 into C3H, and E 0771 into C3H/Jax, A/Heston, and A/Wooley mice. All were sacrificed on an average of 16 days after transplantation.

Gomori's method for alkaline phosphatase activity was used. The seven incubation times used were 1 minute, 5 minutes, 15 minutes, and 1 hour, and, in the case of tumor 15091a, $1\frac{1}{2}$, 2, and $2\frac{1}{2}$ hours. Reaction intensities were visually graded from 0 to 6.

Marked differences were present both in relative phosphatase activity and distribution. Tumor E 0771 had the most activity, beginning at 1 minute, followed by DbrB, which was positive at 15 minutes. Tumor 15091a exhibited no reaction even after $2\frac{1}{2}$ hours.

No differences were observed in transplanting E 0771 into "foreign" strains as C3H/Jax. The intracellular distribution was similar in the series using E 0771 and DbrB where only tumor cells showed activity. Tumor H 2712 revealed a different distribution as luminal borders and vascular channels reacted actively.

Experiments revealed marked variation in phosphatase activity of these four tumors. The enzyme seems inherent in the tumor. Variations in the intracellular distribution were present.

ACTIONS OF 2,4-DIAMINO-5-(3′,4′-DICHLOROPHENYL)-6-METHYLPYRIMIDINE IN MAMMALS. Frederick S. Philips, Leonard D. Hamilton,* Donald A. Clarke,* Stephen S. Sternberg,* and George H. Hitchings. (Division of Experimental Chemotherapy, Sloan-Kettering Institute, New York, and Department of Biochemistry, The Wellcome Research Laboratories, Tuckahoe, N.Y.)

The inhibition of Sarcoma 180 by 2,4-diamino-5-(3′,4′-dichlorophenyl)-6-methylpyrimidine (Clarke *et al.*), previously found to be a folic acid antagonist for *L. casei* (Russell and Hitchings), led to a study of its actions in dogs, cats, rats, and mice. Single parenteral doses of the order of the LD_{50} of the pyrimidine cause convulsions; this is a property common to 5- or 6-alkyl or -aryl substituted 2,4-diaminopyrimidines. Delayed signs of intoxication included occasional emesis, anorexia, loss of weight, leukopenia, reticulopenia, hemoconcentration, and diarrhea (often hemorrhagic). In the rat leukopenia and reticulopenia were associated with hypoplasia of bone marrow; in the marrow of the dog the nucleated erythroid elements underwent "megaloblastic" transformation, and chromosomal aberrations were frequent. Degenerative changes were noted in intestinal epithelium. The pathological changes in mammals resemble those produced by 4-amino analogs of folic acid.

EXFOLIATIVE CYTOLOGICAL DIAGNOSIS OF LESIONS IN THE CENTRAL NERVOUS SYSTEM.

William R. Platt. (University of Pennsylvania School of Medicine, Philadelphia, Pa., and West Jersey Hospital, Camden, N.J.)

Primary or secondary malignant tumors or other masses situated in the brain and spinal cord and encroaching on any surface bathed with cerebrospinal fluid may liberate tumor cells or other cellular fragments into this field. Occasionally, these free-surface desquamations implant themselves to produce metastases on near or distant surfaces of the meninges and ventricles and thereby act as a secondary source of exfoliated cells in the ventricular and subarachnoid spaces. Furthermore, because of the common neurosurgical procedure of needle exploration and aspiration in the region of the cerebral space-occupying mass (in order to ascertain the presence of a tumor, a localized inflammatory or hemorrhagic process, and/or cyst formation in a neoplastic, degenerative, or parasitic process), the Papanicolaou technic was applied also to the fluids obtained therefrom. Microscopic specimens of fluid from ventricular, cisternal, spinal, and cerebral cysts with normal or pathologic cytological structure are presented. The procedure is offered as another diagnostic adjunct to the armamentarium of the neurologist, neurosurgeon, and neuropathologist. The test is not intended to supplant or replace the subjective and objective findings and other diagnostic means utilized by the roentgenologist, neurologist, and neurosurgeon in the examination of the patient with a suspected lesion of the central nervous system.

PROGRESSIVE MICROSCOPIC ALTERATIONS IN THE LIVERS OF RATS FED THE HEPATIC CARCINOGENS 3′-METHYL-4-DIMETHYLAMINOAZOBENZENE AND 4′-FLUORO-4-DIMETHYLAMINOAZOBENZENE.

J. M. Price and J. W. Harman.* (McArdle Memorial Laboratory and Department of Pathology, University of Wisconsin, the Medical School, Madison 6, Wis.)

The progressive microscopic alterations in the livers of rats fed 4-dimethylaminoazobenzene have been thoroughly studied. Recent observations indicated that it might be profitable to investigate the changes produced in the livers of rats by two more potent carcinogens.

Rats were fed a basal diet with or without 0.058 per cent of either 3′-methyl-4-dimethylaminoazobenzene (3′-Me-DAB) or 4′-fluoro-4-dimethylaminoazobenzene (4′-F-DAB), and animals from all groups were selected for histological studies of their livers at frequent intervals until neoplasms were grossly evident. After 5 weeks one group of animals ingesting 3′-Me-DAB was placed on the basal diet to observe the reversibility of the changes. Protein-bound dye was determined in all livers.

Hyaline cytoplasmic inclusions appeared after 7 days of ingestion of either carcinogen, increased in numbers and size to a maximum when the protein-bound dye was present in highest concentration, and then gradually disappeared as the protein-bound dye level decreased. These inclusions were peripheral with 3′-Me-DAB and centrolobular when 4′-F-DAB was fed.

With 3′-Me-DAB a remarkable proliferation of the bile duct epithelium occurred within 3–4 weeks, and most of these cells suddenly disappeared after 6–7 weeks whether the carcinogen was fed or not. Evidence was obtained which suggested that most of these cells may have been converted into parenchymal cells.

Both carcinogens produced neoplasms similar to those induced by 4-dimethylaminoazobenzene. It appeared that the tumors, regardless of their histological appearance, arose from areas of benign bile duct adenomas or cholangiofibrosis.

RIBONUCLEIC AND DESOXYRIBONUCLEIC ACID IN THE NUCLEI AND CYTOPLASMIC FRACTIONS OF RAT

LIVER. I. LEVELS DURING PRE-HEPATOMA PHASE OF DAB FEEDING. J. PRYOR,* G. Z. WILLIAMS, and J. ATKINSON.* (Department of Oncology, Medical College of Virginia, Richmond, Va.)

Sprague-Dawley albino rats have been maintained on synthetic riboflavin-deficient diets containing 0.04 per cent and 0.06 per cent 3′-methyl-4-dimethylaminoazobenzene for periods of 1–20 weeks. At 2-week intervals, a group of rats was sacrificed, and the pooled livers homogenized and fractionated by centrifugation. Total nitrogen and phosphorus were determined on all fractions. Nuclear extracts were analyzed for phosphorus, nitrogen, PNA, and DNA. The levels of pentosenucleic acid and desoxypentosenucleic acid were determined by ultraviolet spectrophotometry after extraction by the Schmidt-Thannhauser modification of the Schneider method. Control animals were also studied after similar periods on the basal diet alone without the azo dye. The results are expressed in μg. per nucleus and per cell. The number of cells was calculated on the basis of the number of nuclei on the same sample, and the nuclei were counted under the phase microscope. The values for DNA and RNA in the cell fractions which were obtained after various periods of basal diet and dye feeding will be compared.

MACROMOLECULAR PARTICLES OBTAINED FROM HUMAN NEOPLASTIC AND NON-NEOPLASTIC LYMPH NODES. III. ELECTROPHORETIC STUDIES. JACOB G. RABATIN,* QUENTIN VAN WINKLE,* MIRIAM S. HOSTER,* and MARIE H. GREIDER* (introduced by Hans G. Schlumberger). (Division of Cancer Research, College of Medicine, The Ohio State University, Columbus 15, Ohio.)

Comparative electrophoretic studies were conducted with the high gravity (25,000 *g*) centrifugation sediments obtained from seven Hodgkin's disease, six lymphatic leukemia, two reticulum-cell sarcoma, two lymphosarcoma, three metastatic carcinoma, and two non-neoplastic lymph nodes.

The electrophoretic analyses were carried out in 0.1 M phosphate buffer, pH 8.0. The following electrophoretic components were present: (*a*) a component exhibiting a mobility of $6\text{–}8\times10^{-5}$ cm^2/volt/sec, which was found in all samples studied; (*b*) a component exhibiting a mobility of $15.5\text{–}18\times10^{-5}$ cm^2/v/sec, which was present in a majority of the preparations; and (*c*) a component exhibiting a mobility of $10\text{–}11\times10^{-5}$ cm^2/v/sec, which was present in approximately one-third of the preparations.

The high gravity sediments obtained from Hodgkin's disease lymph nodes exhibited the greatest variation in the electrophoretic properties. The reticulum-cell sarcoma group and the lymphosarcoma group contained electrophoretic patterns which were similar to each other. High gravity sediments obtained from lymphatic leukemia lymph nodes were characterized by the presence of two electrophoretic components and exhibited the most similar electrophoretic properties of all lymph nodes studied.

The studies concerning mobility as a function of the pH indicate that the particles found in the high gravity sediments have complex surfaces which contain a highly acidic constituent.

DIFFERENCES IN MAMMARY GLAND DEVELOPMENT AMONG DIFFERENT MOUSE STRAINS AS MEASURED BY THE IRON CONTENT OF THE GLAND. H. E. RAWLINSON and G. B. PIERCE.* (Department of Anatomy, University of Alberta, Edmonton, Canada.)

The histologically visible iron that accumulates in the resting mammary glands of mice has been used as a basis for a combined histological and chemical measurement of the differences in glandular development between C3H virgin and breeder females (H. E. Rawlinson and G. B. Pierce, Endocrinology, **46:** 426–33). This study has been extended to cover two other strains, A and C57 black. Throughout the first 10 months of life the total iron, as well as the iron per gram of gland, is greatest in C3H and A breeders, roughly equal in C3H and A virgins, and least in C57 blacks. A marked breeder:virgin difference exists in all three strains. The highest values occurred in C3H mice that had developed tumors.

Estrogen administration influences iron deposition in the gland, and an attempt has been made to assess the effect of pellet implantation in the different strains under varying conditions of castration and ovarian grafting.

SECRETION BY THE STOMACH OF COMPOUNDS DERIVED FROM 2-AMINOFLUORENE. FRANCIS E. RAY and JOHN H. PETERS.* (Cancer Research Laboratory, University of Florida, Gainesville, Fla.)

The carcinogen, 2-aminofluorene, was diazotized and coupled with dimethylaniline, phenol, resorcinol, *m*-cresol, *m*-phenylenediamine, 2,4-diaminotoluene, and *m*-aminophenol. Previous work on the secretion of dyes by the stomach indicated

that compounds with a pK_B of 8 to 10.8 probably would be secreted in substantial amounts.

The resorcinol derivative had a pK_B of 10.4, the *m*-cresol derivative, 10.3, and the 2,4-diaminotoluene derivative, 8.5. The resorcinol and the 2,4-diaminotoluene derivatives were injected into albino rats that had been previously ligated below the pylorus to prevent regurgitation from the intestine.

The 2,4-diaminotoluene derivative which had the most promising pK_B value showed no secretion in 15, 30, or 60 minutes. The resorcinol derivative was secreted to the extent of 30, 28, and 34 per cent of the injected dose in 15, 30, and 60 minutes. The *m*-cresol derivative was then injected. It was secreted to the extent of 24 and 26 per cent in 30 and 60 minutes. One group of rats was painted, and another was given intraperitoneal injections of solutions of the resorcinol derivative. Gastric lesions developed in 7/12 of the first group and 8/12 of the second.

There has thus been obtained for the first time a compound derived from a carcinogen that is secreted in substantial amounts by the stomach and that seems to attack the glandular mucosa. If it proves approximately as carcinogenic as 2-aminofluorene, it has a reasonable prospect of causing gastric tumors.

EFFECTS OF SERUM FROM CARCINOMATOUS MICE ON ERYTHROCYTE METABOLISM. Allen F. Reid, Margaret C. Robbins,* Robert G. Budd,* and Ellsie B. Ryan.* (Department of Biophysics, Southwestern Medical School, University of Texas, Dallas, Texas.)

Some studies were made on the relative effects of normal and cancerous mouse serum on the metabolic phosphate-loss rate of mouse erythrocytes. It has been demonstrated that glucose and an unidentified substance (*s*) contained in serum markedly retard that loss rate. This inhibition has been shown to be less in the case of erythrocytes from mice with large mammary carcinomas (Cancer Research, **4**: 1028, 1944).

With the same technic, erythrocytes were incubated with $P^{32}O_4$-containing serum from both normal and cancerous mice of the same inbred strain. Preliminary experiments indicated less inhibition of phosphate loss where cancerous serum was used. The glucose concentration of the sera was approximately the same.

Erythrocytes were incubated at 37° C. for 5 hours with portions of the same cancer and normal sera. The erythrocytes incubated with cancer serum showed less response to inhibition by glucose and the serum inhibitor than those incubated with normal serum. The results suggest that the primary difference between the blood of the cancerous and that of normal mice is a relative lack of the unidentified inhibitor in the former. With this environmental lack *in vivo*, the cells soon become less responsive to normal metabolic inhibition.

IN VIVO INHIBITION OF SUCCINOXIDASE ACTIVITY IN TISSUES OF NORMAL AND TUMOR-BEARING RATS BY ANTIMYCIN A. Arnold E. Reif* and Van R. Potter. (McArdle Memorial Laboratory, University of Wisconsin, Madison, Wis.)

Previous *in vitro* studies by Potter and Reif (J. Biol. Chem. [in press]) indicated that antimycin A is a powerful inhibitor of an enzyme involved in electron transport. Studies have now been done to test the concept of Ackermann and Potter that a suitable inhibitor of high specificity and "titrating" capacity might be used *in vivo* to inactivate a tissue low in the affected enzyme without seriously affecting tissues rich in the enzyme. This concept may have practical significance in the chemotherapy of cancer.

Succinoxidase activities of various rat tissues were studied with respect to the degree of inhibition by antimycin A. The LD_{50} by intraperitoneal injection was 0.81 mg/kg. At 1 mg/kg, death usually occurred within 1 hour, and at this time the succinoxidase activities of heart, brain, skeletal muscle, thymus, Flexner-Jobling carcinoma, and Walker 256 carcino-sarcoma were essentially unaffected, while those of liver, lung, spleen, and Jensen sarcoma were reduced to 40, 26, 11, and 14 per cent of control values. At higher doses, most tissues showed increased inhibition; brain was unique in remaining entirely unaffected. At a sublethal dose of 0.6 mg/kg, the succinoxidase activities of liver, spleen, and lung fell to 60, 35, and 14 per cent of control within 15 minutes after injection, but returned to normal values within 3 hours. Intravenous injections at 1 mg/kg gave similar effects, but a higher degree of inhibition than intraperitoneal injections at this dose.

The results are examined in terms of enzymatic content, blood supply, and permeability of the tested tissues.

THE CYTOLOGIC SMEAR STUDY OF LIVER NUCLEAR CHANGE IN RATS FED AZO DYE 3′-METHYL-4-DIMETHYLAMINOAZOBENZENE. Howard L. Rich-

ARDSON* (introduced by A. C. Griffin). (University of Oregon Medical School Portland, Ore.)

Rat liver smears prepared during the period of chemical carcinogenic induction revealed unusual chromatin and nucleoli phenomena. This was observed during a 21-week period in which animals were fed a basal diet containing 0.06 per cent 3′-methyl-4-dimethylaminoazobenzene. Liver needle puncture biopsies made possible weekly comparisons of cytologic and histologic material.

Combinations of fixatives were used in the study. Vandegrift's solution proved to be most reliable in fixing liver chromatin and nuclear structure. Ehrlich's hematoxylin, eosin, and orange G were used for staining.

The first cytologic change, observed during the 4 weeks after beginning the 3′-Me-DAB feeding, consisted of enlargement of the liver-cell nuclei with an increase in nuclear chromatin. Regression in cell size, except in isolated clusters, became apparent between the sixth and ninth weeks. The nuclei of the cells in these focal clusters continued to increase in size and to amass chromatin. The nucleoli dispersed, became multiple, and by the twelfth to the fourteenth weeks these cells were 3 times their normal size. Liver punctures at this time were followed by growth of liver cancers of either the large or small cell type along the needle puncture tract. In liver cancers a correlation was noted between nucleoli size and the decrease of nuclear chromatin.

INHIBITION OF CYTOCHROME OXIDASE AND DOPA OXIDASE BY MOUSE MELANOMA EXTRACT.

VERNON RILEY, DEAN BURK, and GEORGE HOBBY.* (National Cancer Institute, Bethesda, Md.)

The presence of naturally occurring inhibitors of two oxidative enzymes has been observed by various technics. An inhibition of the cytochrome oxidase system, amounting to as much as 70 per cent of the potential enzyme activity in the cytoplasmic extracts of mouse melanomas has been observed. The influence of the inhibitor on the enzyme is greatest in concentrated extract and is diminished upon dilution of the extract, which results in a concave downward activity curve. The inhibitor is heat-labile and appears nondialyzable.

An enhancement of the dopa oxidase activity of melanoma extracts was obtained by isolating the enzyme-carrying particulates of the extract by means of a chromatographic system composed of diatomaceous earth and "physiological" solvents. Relative activity of the enzyme was increased more than tenfold over the starting extract, per unit of nitrogen, with no change in the succinoxidase activity used as a basis of control. The dopa oxidase activity, $QO_2(N)$, of the chromatographically isolated granules was approximately fourfold higher than those granules simultaneously purified by differential centrifugation. The data indicate that the observed enhancement of the enzyme is a consequence of the removal of an inhibitor by the chromatographic procedure.

The possible influence of such natural inhibitors in affecting the metabolism of malignant tissue is considered.

A HISTOLOGICAL STUDY OF THE HYPERPLASIA PRODUCED BY CROTON OIL UNDER VARIOUS CIRCUMSTANCES.

A. C. RITCHIE,* E. P. LEROY,* and PHILIPPE SHUBIK. (Departments of Oncology and Pathology, Chicago Medical School, and Mount Sinai Hospital, Chicago, Ill.)

A single application of a carcinogenic hydrocarbon can so change the skin of the mouse that subsequent applications of the noncarcinogenic substance croton oil are enabled to incite tumors in the prepared area. Certain workers have claimed that the hyperplasia produced in mouse skin by such carcinogenic compounds can be distinguished histologically from hyperplasias produced in other ways. The first part of this study compares the histological picture seen when croton oil is applied repeatedly to skin prepared by a single application of carcinogen to that seen when the oil is applied to normal skin.

It has been suggested that, though cortisone does not affect the hyperplasia produced by applications of carcinogen, it greatly modifies that produced by croton oil. In the second part of this presentation the effects of cortisone on the hyperplasia produced by croton oil will be described. Both the findings when croton oil was applied to normal skin and those when it was applied to skin prepared by a previous application of carcinogen will be considered.

INHIBITION OF UTILIZATION OF GLUTAMIC ACID AND GLUTAMINE.

EUGENE ROBERTS and PADMASINI AYENGAR.* (Wernse Laboratory of Cancer Research, Washington University School of Medicine, St. Louis, Mo.)

One of the most interesting findings of an extensive survey of free amino acids in normal and neoplastic tissues of mice was that the glutamine levels in the tumors were uniformly lower than in

most of the normal tissues. It appeared feasible to attempt to disturb the protein synthetic mechanisms in tumors by interference with glutamine metabolism. Most normal tissues, which have relatively large amounts of free glutamine, would presumably be more resistant to such an interference. Glutamine metabolism could be disturbed by inhibiting its synthesis or by blocking its utilization in further metabolic processes. A series of compounds structurally related to glutamic acid and glutamine (obtained through the courtesy of Dr. Karl Pfister of the Research Laboratories of Merck and Co., Inc.) was tested for inhibition in suitable test systems with *Lactobacillus arabinosus* 17–5. DL-α-Methylglutamic acid and one of the diastereoisomeric racemates of β-hydroxyglutamic acid were found to be the most potent inhibitors of glutamic acid utilization yet described. These substances prevented the synthesis of glutamine from glutamic acid by the bacteria. Some details of the effects of these compounds in bacteria and mice and on isolated enzyme systems will be discussed. Although none of the compounds tested to date has proved to be a sufficiently potent glutamine inhibitor, the search for such materials is being continued.

SERIAL TRANSPLANTATION OF VIRUS PAPILLOMAS TO NEWBORN RABBITS. STANFIELD ROGERS* (introduced by Peyton Rous). (The Rockefeller Institute for Medical Research, New York 21, N.Y.)

Two papillomas induced with the Shope virus in adult domestic rabbits were propagated in the leg muscles of several successive groups of newborn animals. In some the papillomatous tissue grew vigorously, forming huge masses that caused early death, in others more slowly, but steadily for months after its hosts had matured. Yet it gained nothing in transplantability from its sojourn in adults, regularly failing when passed on to others. Almost certainly it could have been maintained indefinitely in sucklings.

The transplanted papillomas often entered lymph and blood vessels but never metastasized. Sometimes they penetrated through the reactive tissues and encapsulated them, and on reaching the muscle they replaced fibers individually. In these respects the growths resembled those resulting from auto-implantation in the muscles of adults of papillomas stimulated by infection with bacteria. And indeed both of the growths propagated serially in sucklings contained bacteria and showed signs of their influence.

No carcinomas arose from the papillomatous tissue, though the growths resulting from its repeated transfer were numerous, and it had sometimes proliferated actively for 10–12 months. In contrast, cancers developed within 6–10 months in eight of fifteen rabbits carrying papillomas produced by the same virus materials and situated on the skin. But the intramuscular growths had been protected from trauma.

The successful transplantation of the papillomas adds yet another attribute to those demonstrating that in all immediate ways these growths behave as if neoplastic.

OBSERVATIONS ON THE TRYPTOPHAN PERCHLORIC ACID REACTION OF CANCER SERA. OTTO ROSENTHAL, CHARLOTTE WITMER,* and PEGGY BEATTY.* (Harrison Dept. of Surgical Research, School of Medicine, University of Pennsylvania, Philadelphia, Pa.)

Several tests have been proposed to utilize polysaccharide levels of sera for diagnostic and prognostic purposes in neoplastic diseases. Experiments of Seibert indicated that the colorimetric tryptophan perchloric acid reaction provided a simple and adequate method of analysis. Results from this and other laboratories confirmed the fact that the reaction was generally increased in cancer sera. What constituent of serum was reacting remained obscure. The reaction is almost specific for desoxyribonucleic acid (DNA). Under our test conditions, fructose, sorbose, and fucose yielded, respectively, 44, 24, and 10 per cent, and glucose, galactose, and mannose, common constituents of polysaccharides, less than 1 per cent of the optical density produced by equimolecular amounts of DNA. When the increments in optical density, ΔR, due to the addition of these sugars to serum, were compared with the readings, R, in absence of serum, it was found that the expected ratio, $\Delta R/R=1$, was obtained only with DNA. With all other sugars the ratio approximated 3. Omission of tryptophan from the serum system reduced the ratio to 0.2 and 2.0 with DNA and fructose, respectively. Thus, many sugars, with the exception of DNA, apparently form colored derivatives with serum components other than tryptophan. Since, in routine tests upon normal and cancer sera, omission of tryptophan reduced color formation by but 10 per cent, it seems probable that tryptophan plays only a minor role in Seibert's test.

THE OCCURRENCE IN TARRED RABBIT SKIN OF MINOR, ALMOST IMPER-

CEPTIBLE, NEOPLASTIC CHANGES. PEYTON ROUS and STANFIELD ROGERS.* (The Rockefeller Institute for Medical Research, New York 21, N.Y.)

Previous work in this laboratory has shown that most of the tumors that tar elicits from rabbit epidermis require aid for their survival, which tarring supplies. It brings into existence numerous latent tumor cells which never form growths unless stimulated. The question arises whether some hidden neoplastic elements may not be so poorly endowed as to multiply into visibility only on prolonged urging. The noncarcinogenic stimulus of wound healing was used to find out.

Holes nearly a centimeter across were punched through rabbits' ears, repeatedly tarred months before, and, when they had filled with new tissue, all of this save a peripheral zone was punched out if no tumors had appeared. This was done again and again after healing, only the original zone remaining. Its cells were thus kept multiplying throughout many months, and sooner or later some of them slowly formed small growths. None appeared carcinomatous; most were low papillomas with epithelium that looked merely hyperplastic, while other were of kinds unreported previously. All arose as radial entities on the peripheral zone, extending inwards as the adjacent tissue did so. Their radial shape showed they had originated from cells permanently changed prior to the first punching. The majority soon retrogressed, unless repunching was done.

The experiments have disclosed neoplastic deviations from the normal so slight in their expressions as to become manifest only under exceptional circumstances. They must be reckoned with when considering the nature of neoplastic change.

THE INFLUENCE OF DIETARY 2-ACETAMINOFLUORENE ON RAT LIVER COMPOSITION AND ON *IN VITRO* UPTAKE OF ALANINE-C^{14}. ROBERT J. RUTMAN,* A. CANTAROW, and KARL E. PASCHKIS. (Jefferson Medical College, Philadelphia, Pa.)

The course of 2-acetaminofluorene carcinogenesis in rat liver is accompanied by changes in the quantity of nucleic acids and proteins, as well as in the ratio of these constituents, in the cell components isolated by isotonic sucrose fractionation. Increases in the pentosenucleic acid and protein content of the nuclear fraction and decreases in these constituents in the mitochondria were apparent after 5 weeks of treatment and well established in 10–12 weeks. In the tumor-bearing liver, the mitochondrial protein had been reduced from a normal value of 29.8 ± 0.5 per cent of the tissue to approximately 15 per cent, while the 20 per cent of liver protein normally associated with the nuclear fraction had increased to 35 per cent. The percentage of cell constituents in the supernatant, containing the microsomes, was relatively unaltered.

Changes in the pentosenucleic acid distribution paralleled those in protein. There was some tendency for preferential loss of protein, particularly from mitochondria, resulting in an increased nucleic acid/protein ratio. On the other hand, significant changes in the desoxypentosenucleic acid content were not noted in the precarcinogenic period. In the tumor-bearing livers, DNA increases were inconstant and significant in only a minority of the specimens.

The composition studies were accompanied by tests of the *in vitro* incorporation of alanine-C^{14} into crude mitochondria obtained from normal, pre-tumor, and tumor livers. Comparative assays showed sharp increases in the incorporation of radioactivity, approximating 50 per cent at 5 weeks, 65 per cent at 10 weeks, and 150 per cent at 20 weeks after the start of 2-acetaminofluorene treatment.

STUDIES ON THE EARLY STAGES OF MEDIASTINAL LYMPHOSARCOMA IN C57 MICE. JORGEN RYGAARD (introduced by W. U. Gardner). (Department of Anatomy, Yale University School of Medicine, New Haven, Conn.)

Mice of several strains develop leukemia after exposure to x-radiation in proper doses (Krebs, 1930; Furth, 1934; Henshaw, 1944). Some of these strains such as BC and C57 develop anterior mediastinal lymphosarcoma.

Two hundred and fifty-five mice of the BC strain were given 285–380 r single dose total-body irradiation alone or plus estrogens or androgens. Sixty-one developed mediastinal lymphosarcoma, all with a peculiar pattern of the reticular fibers. The tumors were fixed in Bouin's solution, and paraffin sections were stained with hematoxylin, eosin, and by Laidlaw's silver method.

In the tumors a condensation of reticular fibers was found surrounding a central area, which was free of reticular fibers except along blood vessels. In sharp contrast to this internal area, the peripheral portion of the tumors had numerous evenly dispersed reticular fibers.

The same pattern was found in x-ray-induced mediastinal lymphosarcoma of C57 mice but was

not found in normal thymuses, nontumorous x-radiated thymuses, or metastases from mediastinal lymphosarcoma.

In order to see how early in the tumorigenesis the structure of the thymus changes, C57 mice were given 80 r total-body irradiation on each of 12 consecutive days. Mice were killed at intervals after irradiation from 1 to 4½ months. In all enlarged thymuses and in several grossly normal ones this characteristic pattern of reticulum was found.

INHIBITION OF AZO DYE CARCINOGENESIS IN THE ALLOXAN-DIABETIC RAT.

David A. Salzberg* and A. Clark Griffin. (Department of Chemistry, Stanford University, Stanford, Calif.)

This paper presents a new finding concerning the effect of the carcinogenic azo dye, 3′-methyl-4-dimethylaminoazobenzene (3′-Me-DAB) on white rats previously made diabetic by the injection of alloxan. One week after alloxan administration, groups of rats were fed ad libitum on a semi-synthetic diet containing 0.06 per cent of 3′-Me-DAB. This diet was maintained for 3 months, and thereafter the animals were fed Purina Laboratory Chow with no further administration of the azo dye.

Four to 8 weeks after discontinuance of the carcinogen, the animals were sacrificed or laparotomized and inspected for hepatoma development. Of a group of fourteen animals, one showed evidence of liver tumor, two had moderate liver necrosis, and the rest had smooth-surfaced, normal-appearing livers. Comparable groups of control animals exhibited 90–100 per cent of hepatoma incidence. It should be noted that the increased food intake of the diabetic animals results in the ingestion of approximately twice the quantity of azo dye ingested by the control dye-fed animals. Microscopic examination of sections from the normal-appearing livers of the diabetic animals showed a few small areas of hyperplasia, possibly comparable to the very early stages of azo dye carcinogenesis after 2 or 3 weeks of feeding of the dye.

A comparison is made of the nucleic acid, riboflavin, and bound dye content of the livers from the azo dye-fed diabetic animals and diabetic and normal control animals.

THE CELLULAR RESPONSE TO METHYLCHOLANTHRENE AND TO TALC IN THE BODY CAVITY OF THE COCKROACH *PERIPLANETA AMERICANA*.

Hans G. Schlumberger. (Department of Pathology, College of Medicine, Ohio State University, Columbus, Ohio.)

Crystals of methylcholanthrene or talc were introduced into the body cavity of cockroaches through a small incision on the dorsal or ventral surface of the abdomen. The animals were sacrificed 7 hours to 365 days later and the tissues examined microscopically. No difference was noted between the response to methylcholanthrene and to talc; nor did the site of the injury, whether dorsal or ventral, alter the course of the reaction. Within 7 hours hemocytes were present in large numbers about the crystals; subsequently, more hemocytes appeared, and the innermost became fusiform. Frequently the response was most marked about the larger tracheae. The tracheal cells rounded up and were difficult to distinguish from hemocytes; the tracheal lumina often were plugged with chitin. Even when larger tracheae were absent, small radicals penetrated the cell masses in all directions, resembling in their size and distribution the capillaries seen in the granulation tissue of vertebrates. Frequently, the wall of the midgut was the seat of focal necrosis; these areas were surrounded by the most massive cellular response observed in this study. In several instances there was a distinct histological resemblance to the connective tissue neoplasms of vertebrates; however, in no case was there evidence of autonomous growth. The lesions emphasize the difficulty often encountered in distinguishing between reactive hyperplasia and true neoplasia.

ELECTROPHORETIC ANALYSIS OF THE SERUM OF RATS BEARING TUMORS INDUCED BY GASTRIC INSTILLATION OF METHYLCHOLANTHRENE.

Julius Schultz,* Harry Shay, William Jamison,* and Margot Gruenstein.* (Samuel S. Fels Research Institute, Temple University School of Medicine, Philadelphia, Pa.)

The successful production of adenocarcinoma in the rat by gastric instillation of methylcholanthrene (Shay *et al.*, J. Nat. Can. Inst., **10**: 255, 1949) provides a readily reproducible means for the study of induced cancer. As part of a program for the biochemical characterization of this animal we have examined the proteins of the sera by electrophoretic analysis.

In these studies the sera of one to three rats were pooled for each analysis. Eight such pools were used for the normal group (N), seven for the tumor-bearing rats (TB), and six for the methyl-

cholanthrene-fed rats (MC) without tumors. The values for the percentage of the total area for albumin, alpha-1 globulin, alpha-2 globulin, beta-globulin, and gamma-globulin were: for group (N), 48.6 ± 1.7, 13.7 ± 3.0, 10.0 ± 1.3, 14.4 ± 1.2, and 13.2 ± 3.0, respectively; for group (TB), 47 ± 5.6, 13.8 ± 3.0, 10.9 ± 2.0, 19 ± 3.2, and 9.1 ± 0.7; for (MC), 48 ± 2.3, 15 ± 2.9, 8.9 ± 1.3, 16.9 ± 1.4, and 11.1 ± 1.8. A splitting of the beta-globulin was found in five pools of the tumor-bearers and four pools of the MC group. The slower component of these comprised 11.9 ± 0.9 and 11.2 ± 1.2 per cent of the total area in each case.

Recent reports indicate that in cancerous mouse sera, rat plasma, and human sera there is a depression of the gamma-globulin and in some cases of albumin; the significance of the elevated beta-globulin and the splitting of this fraction found here will be discussed in relation to these reports.

EFFECT OF 2-CHLORO-2′-HYDROXYDIETHYL SULFIDE (HEMISULFUR MUSTARD) ON CARCINOMATOSIS WITH ASCITES. Arnold M. Seligman, Alexander M. Rutenburg, Lester Persky,* and Orrie M. Friedman.* (Harvard University and Beth Israel Hospital, Boston, Mass.)

A preparation of 2-chloro-2′-hydroxydiethyl sulfide (hemisulfur mustard, HSM) free of traces of mustard gas is $\frac{1}{3}$ as toxic as methylbis(β-chloroethyl)amine hydrochloride in mice.

The toxic reactions seen after intravenous administration in human subjects regularly consisted of nausea, vomiting, malaise and weakness, and, rarely, of tremor and convulsions. Blood cell counts were not affected. HSM produced inflammation and thrombosis of the vein through which it was administered.

Of 31 patients with malignant disease, ten patients appeared to be temporarily benefited by HSM. Two of these had prostatic carcinoma with metastases, and eight had ascites from peritoneal carcinomatosis. Palliation consisted in decreased formation of ascites, decrease in pain, increased appetite, strength, and ability to get around. Two patients showed a decline in serum acid phosphatase. The longest remission of symptoms was 10 months, and the shortest was several weeks.

PLASMA SULFHYDRYL LEVELS DURING TUMOR GROWTH. Bernard Shacter, Cecil Entenman,* and Michael B. Shimkin. (Division of Biological and Medical Sciences, U.S. Naval Radiological Defense Laboratory, and of the Laboratory of Experimental Oncology, National Cancer Institute, and the Division of Medicine, University of California School of Medicine, San Francisco, Calif.)

In experiments previously reported (B. Shacter, Cancer Research, **11**: 277, 1951), sulfhydryl levels of rat plasma were shown to decrease after surgical trauma or exposure of rats to x-radiation. It was suggested that the changes were related to increased tissue requirements for sulfhydryl groups for cell growth and division during the recovery process. The experiments have now been extended to determine the effect of tumor growth on plasma sulfhydryl levels.

Murphy rat lymphosarcoma was transplanted subcutaneously into Sprague-Dawley rats, and amperometric sulfhydryl determinations were carried out on the plasma at intervals thereafter. Within 2 days after transplantation, there was an initial, nonspecific decrease in plasma sulfhydryl. The concentration began returning toward normal during the lag phase in tumor growth and then decreased sharply as tumor growth became accelerated. With the eventual slackening of the growth rate and ulceration of the tumor, plasma sulfhydryl levels rose again.

During the administration of cortisone to tumor-bearing rats, the growth of the tumor was delayed, and the fall in plasma sulfhydryl levels associated with tumor growth was markedly limited.

The results obtained indicate that plasma sulfhydryl levels vary with the rate of tumor growth. They are consistent with the hypothesis that changes in plasma sulfhydryl levels are related to tissue requirements for sulfhydryl groups.

COMBINATION CANCER CHEMOTHERAPY WITH 8-AZAGUANINE AND SEX HORMONES ON A MOUSE BREAST CANCER. Daniel M. Shapiro. (Department of Surgery, College of Physicians and Surgeons, Columbia University, New York, N.Y.)

Earlier studies have established that the growth of the mammary Adenocarcinoma 755 in C57 mice is inhibited by 8-azaguanine. Data on the effects on this tumor of testosterone and stilbestrol, alone and in combination with 8-azaguanine, will be presented.

Stilbestrol not only inhibits the growth of this tumor but greatly enhances the carcinostatic effect achieved with 8-azaguanine. The marked tumor inhibitory activity of this drug combination is accompanied by weight loss, which can be minimized by the addition of testosterone to the combination.

Testosterone has no effect on the growth of this tumor either alone or in combination with 8-azaguanine. Testosterone can protect against stilbestrol toxicity without interfering with the carcinostatic effect of the female sex hormone.

It appears possible that these studies will lead to far more effective chemotherapy with 8-azaguanine.

TUMOR INCIDENCE IN F_1 AND F_2 GENERATIONS DERIVED FROM FEMALE RATS FED METHYLCHOLANTHRENE BY STOMACH TUBE PRIOR TO CONCEPTION.

HARRY SHAY, MARGOT GRUENSTEIN,* and MORRIS WEINBERGER.* (Samuel S. Fels Research Institute, Temple University School of Medicine, Philadelphia 40, Pa.)

Eighteen female Wistar rats, ranging in age from 35 to 48 days, received 2 mg. of methylcholanthrene in 0.2 cc. of olive oil daily by stomach tube for approximately 2 months. Treatment was discontinued immediately after these female rats were impregnated by untreated normal male rats of comparable age. The offspring of this mating (F_1) consisted of 35 animals, seventeen males and eighteen females. Six of the 35 animals were tumor-bearers (three males and three females) at ages from 9 to 24 months. There was one multiple tumor-bearer. The tumor frequency was 17.1 per cent for the entire group. Pathological analysis revealed three fibroadenomas of the breast, one pituitary adenoma, two reticulum-cell sarcomas, and one lymphosarcoma. Brothers and sisters of F_1 were mated at 4 months of age yielding 88 animals (42 males and 46 females) in the second generation (F_2). Eighteen animals developed tumors (sixteen females and two males at ages from 12.5 to 24.5 months). Four females bore multiple tumors. Tumor frequency for this group was 20.5 per cent. Pathologically, these comprised three adenocarcinomas of the breast, five carcinomas of the uterus, one hepatoma, three reticulum-cell sarcomas, six fibroadenomas of the breast, one thymoma, one lipoma, one chromophobe adenoma of the pituitary, and one papillary cystadenoma of the neck.

Statistical analysis of tumor incidence in F_1 and F_2 revealed a significant increase ($P < 0.01$) over the incidence of spontaneous tumors occurring in the Wistar stock colony.

A FURTHER INVESTIGATION OF MELANOTIC TUMORS IN THE AXOLOTL (*SIREDON MEXICANUM*).

E. A. SHEREMETIEVA-BRUNST (introduced by V. V. Brunst). (Department of Anatomy, University of Maryland Medical School, Baltimore, Md.)

During the period 1939–43, seven successive transplantations of melanotic tumor in axolotl were made. In some cases this transplantable tumor showed extremely active growth which greatly exceeded the growth of all observed spontaneous tumors of the same kind. Cachexia and early death of the animals which received melanoma grafts were evidence of the great malignancy of this tumor. The greater part of this tumor material was lost during World War II. Histological examination of the remaining (salvaged) material, namely, several spontaneous black spots (which sometimes develop into tumors) and several spontaneous melanotic tumors revealed the following: They consist mostly of branching black chromatophores similar to the typical amphibian melanophores. A few contracted melanophores can be found in some portions of these formations. In all cases examined, the transplanted tumors consisted only of contracted black pigment cells. The contraction apparently occurs at the first grafting and is retained through all successive transplantations. This feature can be used to distinguish microscopically spontaneous and transplanted melanomas in the axolotl.

A FURTHER INVESTIGATION OF TUMOR-LIKE STRUCTURES WHICH DEVELOP AFTER IMPLANTATION OF PARTS OF AXOLOTL EMBRYOS INTO THE EYES OR LIMBS OF ADULT AXOLOTLS.

E. A. SHEREMETIEVA-BRUNST,* V. V. BRUNST, and FRANK H. J. FIGGE. (Department of Anatomy, University of Maryland Medical School, Baltimore, Md.)

Different parts of axolotl embryos at a stage just before hatching were implanted into 30 eyes and seventeen limbs of 25 adult axolotls. Among the eye implants, eleven did not grow, nineteen grew, but ten of the latter showed regressive development later and gradually disappeared. Among the limb implants no growth was observed in three cases. In fourteen limbs the implants grew, and regression was observed in only one of these.

In most cases, growing implants formed tumor-like outgrowths. The largest growths measured about 1 cm. in diameter. Some of these regained their size 2, 3, or even 4 times after partial amputation for transplantation of the tissue to new hosts.

Fifty-six transplantations were made from eight of these tumor-like structures which showed

active growth. Only a few transplants increased beyond their original size.

Histological examination of several outgrowths (most of them are kept for further observation of their development) showed that three types of microscopical structures could be distinguished: (*a*) Some of the tumor-like growths consist of an irregular mixture of completely differentiated tissues. (*b*) In spite of the rather advanced age of some of these growths, some portions of them consist of typical embryonic tissue (mesenchyme). (*c*) Some tumor-like structures or parts thereof consist of undifferentiated cells exhibiting great mitotic activity. Histologically they resemble real tumors.

A CONSIDERATION OF CERTAIN DOSAGE-EFFECT RELATIONSHIPS IN CARCINOGENESIS. PHILIPPE SHUBIK and A. C. RITCHIE.* (Department of Oncology, Chicago Medical School, Chicago, Ill.)

In the literature much consideration has been given to the relationship of the dosage of a carcinogen to its effect. In most reports the tumor incidence has been estimated by counting the number of tumor-bearing animals. Recent work suggests that this is an unsatisfactory measure. For example, when tumors are induced by a single application of a carcinogen followed by repeated applications of croton oil, the total number of tumors induced varies semi-logarithmically with the concentration of carcinogen. There is no such relationship between the number of tumor-bearing animals and the concentration of carcinogen.

Experiments have been performed with different numbers of applications of carcinogen, with and without croton oil, and the number of tumors induced in the various experiments compared. Many of the conclusions drawn from experiments in which the number of tumor-bearing animals was considered the important variable have been found misleading. In particular, the concept that a theoretical "100 per cent incidence" is reached, if carcinogen applications are continued long enough, is found meaningless. Further implications are discussed in their bearing on the mechanism of carcinogenesis.

OXIDATIVE RATE AND PHOSPHATE TURNOVER IN HOMOGENATES OF TUMORS. PHILIP SIEKEVITZ,* HERBERT C. SIMONSON,* and VAN R. POTTER. (McArdle Memorial Laboratory, University of Wisconsin, Madison, Wis.)

The addition of fluoride to isotonic homogenates of Flexner-Jobling tumor permits the demonstration of oxidative rates on pyruvate plus fumarate that approximate the rates observed in slices, as reported earlier by Potter and Lyle. The addition of coenzyme I will not compensate for the absence of fluoride in this system. Further studies with added fluoride, dinitrophenol (DNP), calcium ions, or hexokinase, added to both normal and tumor tissues, give additional support to the concept that the rate of phosphorylative oxidation is maximal only when the balance between phosphate donors and phosphate acceptors is optimal. An index of the balance between these factors may be obtained by measuring the ratio of the oxidative rate under standard conditions (Mg, ATP, phosphate, pyruvate, fumarate) to the rate with DNP or with fluoride in addition. Separate studies with rat liver mitochondria show that DNP and fluoride act indirectly to increase and decrease the rate of ATP breakdown, respectively. The balance between phosphorylation and dephosphorylation is different in each tissue, as demonstrated by the effects of fluoride and of DNP on the oxidative rates, and in homogenates of the rapidly growing Flexner-Jobling rat carcinoma the rate of ATP breakdown outpaces ATP resynthesis unless fluoride is added. However, in homogenates of a very slowly growing transplantable mouse tumor taken 4–6 months after implantation, excellent rates of oxidation have repeatedly been observed without fluoride, while fluoride depressed the oxidative rate, suggesting that the phosphate turnover is relatively slow in this tumor.

THE INFLUENCE OF NATURAL FOODS VERSUS SEMI-PURIFIED RATIONS ON THE FORMATION OF TUMORS. HERBERT SILVERSTONE, ROBERT D. SOLOMON,* and ALBERT TANNENBAUM. (Department of Cancer Research, Medical Research Institute, Michael Reese Hospital, Chicago, Ill.)

It is assumed that rations equivalent in caloric content and proportions of adequate protein, fat, vitamins, and minerals, regardless of source, are similar with regard to their influence on the genesis of tumors. This is probably true in general, although there are a few exceptions that have not been explained.

In this respect, the data obtained in a series of experiments are of interest. Spontaneous benign hepatomas developed in relatively low incidence among DBA male mice raised on commercial diets composed principally of natural food (Purina Laboratory Chow, etc.); in contrast, a consider-

ably higher incidence was found in animals fed semi-purified rations composed mainly of casein, cornstarch, partially hydrogenated cottonseed oil, synthetic vitamins, and a salt mixture. Semi-purified rations also enhanced the formation of the spontaneous benign hepatoma of the C3H male mouse. The factors of the experiments indicate that the diversity in tumor incidence was not due to obvious differences in calorie intake or body weight, or proportions of protein, fat, vitamins, and minerals in the diets. In preliminary attempts to find the cause of this arresting difference, the semi-purified ration was supplemented with rutin and ascorbic acid or with liver powder; these had no influence on the incidence of hepatomas.

The significant dependence of the spontaneous benign hepatoma of the mouse on the manner in which the diet is constructed does not imply that other tumor types respond similarly. In comparable experiments with the spontaneous mammary carcinoma and the benzypyrene-induced skin tumor, questionable effects were obtained.

MALIGNANT CHANGES INDUCED IN EXPERIMENTAL ANIMALS BY A MYCOBACTERIUM DERIVED FROM BOTH HUMAN AND ANIMAL TUMORS. Lawrence W. Smith, Virginia Wuerthele Caspe,* and Eleanor Alexander-Jackson.* (Laboratories for the Study of Proliferative Disease, Presbyterian Hospital, Newark, N.J.)

As previously reported, a pleomorphic organism having a filtrable phase, but characteristically showing cultural and morphologic features of the mycobacteria, has been recovered from all types of human and animal malignant tumors as well as from the blood of such subjects. This organism, when injected into experimental animals including mice, guinea pigs, and rabbits, will induce marked proliferative changes which in most instances assume the appearance of a granulomatous type of lesion, morphologically not unlike the tubercle or gumma. In a large proportion of such lesions cells can be found which show nuclear changes strongly suggestive of malignancy. In between 5 and 10 per cent of such cases, frank malignant cell changes occur which may or may not reproduce the type of tumor from which the organism was recovered. More commonly, the lesions are of a sarcomatous nature and most frequently involve the lymphoid and reticuloendothelial tissues. However, carcinoma of the lung, breast, liver, and other organs has been found. An attempt is made in this paper to establish the etiologic basis of the organism in the development of these malignancies.

THE EFFECT OF AN ASCORBIC ACID ANALOG ON ASCORBIC ACID CONTENT AND WHITE BLOOD CELLS OF Ak LEUKEMIC MICE. Boris Sokoloff, Walter H. Eddy, John Beaumont,* Rita Powella,* and George Cone.* (Southern Bio-Research Laboratory, Florida Southern College, Lakeland, Fla.)

The ascorbic acid analog, D-gluco-ascorbic acid, interferes with the synthesis of ascorbic acid in rats and mice. Eighty Ak mice showing symptoms of leukemia were divided into two groups. Fifty mice were placed on a Sherman-LaMer scorbutogenic diet to which 5 per cent of D-gluco-ascorbic acid and 0.5 per cent of flavonoids were added. After 5 days, D-gluco-ascorbic acid was reduced to 3 per cent for 5 days and then to 2 per cent. The ascorbic acid content was reduced to 0.03-0.05 mg/100 cc of plasma and maintained on this level. The number of white blood cells gradually reached a subnormal level of 2,500 (average). The mice remained on this regime for several weeks without any ill effect. Mortality rate was 6 per cent. In the control mice (30) kept on regular diet, all but three died (mortality, 90 per cent). It was found necessary for the well-being of mice not to suppress completely the synthesis of ascorbic acid but to keep its content on a minimal level.

STUDIES OF INTESTINAL ABSORPTION FOLLOWING SMALL BOWEL RESECTION FOR SARCOMA. Herta Spencer* and Isaac Lewin.* (Division of Neoplastic Diseases, Montefiore Hospital, New York, N.Y.)

The mechanism of diarrhea due to apparent faulty intestinal absorption was studied under metabolic conditions in a patient in whom about 80 per cent of the small intestine and the ascending colon had been resected for sarcoma.

Nitrogen, calcium, phosphorus, potassium, and sodium balances were correlated with water and fat contents of stools. The rate of calcium utilization following its oral administration at various levels and following intravenous infusions was studied. Metabolic data and serum electrolyte levels were correlated with the patient's clinical course.

These studies have shown that this patient lost considerable quantities of electrolytes through the intestinal canal and that this loss was in direct relation to the fluid volume of the stool. It appeared that the total electrolyte concentration of the fecal fluid approximated that of a "plasma-ultra-filtrate." Also, the degree of utilization of minerals

and proteins was improved when the intake was increased. This indicated that the remnant of the small intestine had as good an absorptive capacity as the intact intestine. The loss of fluids with the concomitant loss of electrolytes appeared to be the primary cause of the diarrhea. Metabolic studies have also shown that the body is partially able to compensate for losses of electrolytes through the intestinal canal by decreased renal excretion of these substances.

THE EFFECT OF VARYING CONCENTRATIONS ON THE INHIBITION OF CHEMICAL SKIN CARCINOGENESIS IN MICE. D. WARREN STANGER,* WILLARD T. HILL,* ANTHONY PIZZO,* BYRON RIEGEL, and WILLIAM B. WARTMAN. (Departments of Chemistry and Pathology, Northwestern University, Evanston and Chicago, Ill.)

The mean latent period of tumors produced in mice by strong chemical carcinogens has been shown to be prolonged by certain hydrocarbon inhibitors. In the present experiments, the effect of variations in the concentration of a carcinogen (9,10-dimethyl-1,2-benzanthracene) and of an inhibitor (8-methyl-1,2-benzanthracene) was studied. The interscapular skin of groups of 30 CAF_1 hybrid mice was painted twice weekly with acetone solutions of the carcinogen or of carcinogen-inhibitor mixtures.

In one series of experiments, the ratio of carcinogen to inhibitor was kept constant at 1:2, but the concentrations of the two compounds were varied. At the highest carcinogen-inhibitor concentrations, prolongation of mean latent period was much less pronounced than at the lower concentrations.

In another series, the concentration of the carcinogen was kept constant, but the concentration of the inhibitor ranged from zero to 50 times that of the carcinogen. When the concentration of the inhibitor was one-tenth that of the carcinogen, no prolongation of latent period occurred. When the concentration of inhibitor was increased to one-half that of the carcinogen, the mean latent period was increased by about 40 per cent. Progressively greater prolongation of latent period occurred in animals receiving the mixtures containing the inhibitor in still higher concentrations.

The significance of these results will be discussed.

GENETIC STUDY OF NATURAL ANTISHEEP AGGLUTININS IN MICE. KURT STERN and ISRAEL DAVIDSOHN. (Department of Pathology, Chicago Medical School, and Mount Sinai Medical Research Foundation, Chicago, Ill.)

In previous work the authors demonstrated that natural antisheep agglutinins in inbred mice vary considerably in incidence and titer, depending on the strain. This suggested the possibility that these differences may be related to genetic factors. In order to study this possibility, hybridization was carried out between strains C57 black and C3H. In the first strain natural antisheep agglutinins occur in more than 90 per cent with a mean titer of 12.7 in animals having antibodies, whereas less than 30 per cent of the latter strain show presence of these antibodies (mean titer, 2.2). Natural antisheep agglutinins were determined in F_1, F_2, and backcrosses of F_1 to both parental strains. The findings were compatible with the assumption that multifactorial genes (polygenes) determine presence and titer of the antibodies. Further investigations dealt with variations of sheep agglutinins which were found to occur within different sublines of C57 black mice. A possible outcome of this work might be the utilization of antibody levels as a measure of homozygosity in the study of inbred mice.

SELECTIVE LOCALIZATION OF SULFAPYRAZINE IN CANCER TISSUE AFTER ALLOXAN. CHARLES D. STEVENS, PATRICIA M. QUINLIN,* ANNA MARY KOCK,* and MARY ANN WAGNER.* (College of Medicine, University of Cincinnati, Cincinnati 19, Ohio.)

Acidity of cancer tissue can be increased *in vivo* by intraperitoneal glucose injection so that the pH of the cancer tissue drops below 6.4, as measured by glass electrode. This fact may be used to produce high local concentration in cancer tissue of certain types of compounds—compounds less soluble at pH 6.4 (tumor pH) than at pH 7.4 (serum pH). Having found that there is such a selective concentration in the case of sulfapyrazine administered at sites distant from the cancer (Science, **112**: 561, 1950), similar experiments were tried in which blood sugar was elevated by administration of alloxan rather than glucose.

Rats with Walker tumor 256 were starved 2 days, then given a subcutaneous or intraperitoneal injection of Eastman alloxan. After 1 or 2 days they were given a single injection of aqueous sodium sulfapyrazine and were killed 1 or 2 days later. Sulfa concentrations in whole tumors exceeded those in serum from blood drawn at killing in over half the rats for which 2 days elapsed be-

tween alloxan and sulfapyrazine injections, but in only a few of the rats for which 1 day elapsed between alloxan and sulfapyrazine injections. Sulfa concentrations in other tissues were similar to those previously reported.

SYSTEMIC EFFECTS OF TUMORS IN FORCE-FED RATS. II. INFLUENCE OF DIET ON CARCASS COMPOSITION AND PLASMA LIPIDS. A. G. STEWART* and R. W. BEGG* (introduced by J. B. Collip). (Department of Medical Research, University of Western Ontario, London, Canada.)

It has been reported that force-feeding a high fat diet prevents the loss of carcass weight in tumor-bearing rats, and a marked lipemia was noted. This investigation has been extended to include carcass analysis and feeding of different isocaloric diets.

On high fat, carbohydrate, and protein diets loss of carcass weight, nitrogen, and fatty acids is prevented in large measure. The lipemia is marked on the 84 per cent fat diet (caloric), reaching 5 per cent, less on the 60 per cent fat diet, and least on the 60 per cent carbohydrate and 60 per cent protein diets. Phospholipid and cholesterol are increased in proportion to the lipemia. Fatty livers were noted on the original high fat diet, but not with additional choline and the substitution of lactalbumin for albumin.

INHIBITORY ACTION OF SOME NEW PHOSPHORAMIDES ON SARCOMA 180 IN MICE. C. CHESTER STOCK, S. M. BUCKLEY, D. A. CLARKE,* R. P. PARKER,* M. L. CROSSLEY, E. KUH,* and D. R. SEEGER.* (Division of Experimental Chemotherapy, Sloan-Kettering Institute for Cancer Research, New York, N.Y., and Calco Chemical Division, American Cyanamid Company, Bound Brook, N.J.)

Studies stimulated by the effectiveness of 2,4,6-tris(ethylenimino)-*s*-triazine (TEM) against various experimental tumors resulted in the reported finding that two phosphoramides, N,N′,N″-triethylene phosphoramide and N,N-diethyl-N′,N″-diethylene phosphoramide, can markedly retard the growth of Sarcoma 180 in mice. Although their activity is not greater than that of TEM, each of them shows a greater range between the maximum tolerated dose and the minimum dose giving a definite inhibition. Eight related phosphoramides have been synthesized and tested for ability to inhibit the growth of Sarcoma 180 in mice. The variations in their inhibitory activity will be reported.

TWELVE MUTATIONS IN ONE DESCENT OF MICE INJECTED WITH METHYLCHOLANTHRENE. LEONELL C. STRONG. (Yale University School of Medicine, New Haven, Conn.)

The Br descent has been inbred for 35 generations. Both parents of each generation, in the experimental series, since the F_4 have been injected with methylcholanthrene at 60 days of age. The first mutation occurred in the F_{14} (b−B). Between the $F_{14}-F_{35}$, eleven other color mutations have been obtained. (Mutation rate, 12:8,400 mice or 1:700.) The controls, made up of fifteen original inbred strains of mice together with the original Br descent continued free from any injection of methylcholanthrene, have given rise to nine mutations in 232,000 mice (mutation rate in controls, 1:232,000 or 1:23,777 mice). In addition, several phenocopies and somatic mosaics have also occurred in the methylcholanthrene-injected series. Mutations may or may not be accompanied by concomitant changes in susceptibility to chemically induced fibrosarcomas, and changes in susceptibility to methylcholanthrene-induced fibrosarcomas can take place without mutations involving color.

THE EFFECT OF METHYLBIS(β-CHLOROETHYL)AMINE OXIDE, N,N′,N″-TRIETHYLENE PHOSPHORAMIDE AND N,N-DIETHYL-N′,N″-DIETHYLENE PHOSPHORAMIDE ON THE GROWTH OF A VARIETY OF MOUSE AND RAT TUMORS. KANEMATSU SUGIURA and C. CHESTER STOCK. (Division of Experimental Chemotherapy, The Sloan-Kettering Institute for Cancer Research, New York, N.Y.)

This report consists of observations on the effect on the growth of various tumors in animals of methylbis(β-chloroethyl)amine oxide HCl (HN2-oxide),N,N′,N″-triethylene phosphoramide (SK 3818), and N,N-diethyl-N′,N″-diethylene phosphoramide (SK 4614). In general, the first intraperitoneal injection of compounds was given 1–7 days after tumor transplantation, and injections were continued for 7–14 days.

Daily doses of 2 mg/kg of HN2-oxide in mice had a destructive effect on Carcinoma 1025 and Ehrlich carcinoma (ascitic form); a slight inhibitory effect on Sarcoma 180, Miyono carcinoma, and Patterson lymphosarcoma; but no effect on Sarcoma T 241, Sarcoma MA 387, adenocarcinoma E 0771, Bashford carcinoma 63, Ehrlich carcinoma (solid form), Grand epidermoid carcinoma, Wagner and Ridgway osteogenic sarcomas,

Mecca lymphosarcoma, and Harding-Passey melanoma. Daily doses of 1.5 mg/kg of HN2-oxide in rats had a destructive effect on Flexner-Jobling carcinoma and Sarcoma R 39; a moderate inhibitory effect on Walker carcino-sarcoma 256, but no effect on Murphy-Sturm lymphosarcoma.

Daily doses of 6 mg/kg of SK 3818 in mice had a marked inhibitory effect on Carcinoma 1025, Miyono carcinoma, Ehrlich carcinoma (ascitic form) and Ridgway osteogenic sarcoma; a moderate inhibitory effect on adenocarcinoma E 0771, Grand epidermoid carcinoma, and Patterson and Mecca lymphosarcomas; a slight inhibitory effect on Ehrlich carcinoma (solid form); but no effect on Sarcoma 180, Sarcoma T 241, Sarcoma MA 387, Bashford carcinoma 63, Wagner osteogenic sarcoma, and Harding-Passey melanoma. Daily doses of 1 mg/kg of SK 3818 in rats had a destructive effect on Flexner-Jobling carcinoma, Walker carcino-sarcoma 256 and Sarcoma R 39, but no effect on Murphy-Sturm lymphosarcoma.

Daily doses of 4 mg/kg of SK 4614 in mice had a marked inhibitory effect on Carcinoma 1025 and Ridgway osteogenic sarcoma; a moderate inhibitory effect on Bashford carcinoma 63; but no effect on Sarcoma 180, Sarcoma T 241, Sarcoma MA 387, adenocarcinoma E 0771, Miyono carcinoma, Ehrlich carcinoma (solid and ascitic forms), Grand epidermoid carcinoma, Wagner osteogenic sarcoma, Patterson and Mecca lymphosarcomas, and Harding-Passey melanoma. Daily doses of 1 mg/kg of SK 4614 in rats had a destructive effect on Flexner-Jobling carcinoma and Sarcoma R 39; a marked inhibitory effect on Walker carcino-sarcoma 256; but no effect on Murphy-Sturm lymphosarcoma.

A NEW TECHNIC FOR THE INTRA-ARTERIAL ADMINISTRATION OF CHEMOTHERAPEUTIC AGENTS. I. NITROGEN MUSTARD.

ROBERT D. SULLIVAN,* RALPH JONES, JR., JEANNETTE MCC. SHOREY, and TRUMAN G. SCHNABEL, JR.* (C. Willard Robinson Section and the Robinette Foundation of the Department of Medicine, University of Pennsylvania, Philadelphia, Pa.)

A simple method of introducing a small catheter into the arterial tree, developed for studying intra-arterial pressures, has been adapted for the intra-arterial administration of chemotherapeutic agents. This technic has been utilized to study the palliative effect described by Bierman *et al.* and Klopp *et al.* of the intra-arterial administration of nitrogen mustard (HN2) on a variety of different inoperable neoplasms in man.

The technic involves the use of small polyvinyl catheters which are introduced following percutaneous puncture of the peripheral artery with a special #19-21 gauge needle. The needle is then removed, and the catheter may be advanced to a desired site in the central or peripheral vascular tree. The catheters may be left in place for at least 2 weeks, or repeated punctures may be made at the same site. The advantages and limitations of this technic will be discussed.

Regional nitrogen mustard has been administered 48 times in eighteen patients by this technic. Complications have been infrequent. We have confirmed the observation of others that intra-arterial HN2 may modify certain neoplasms which are not affected by intravenous administration of the drug. Hematologic changes were qualitatively similar to those developing with intravenous HN2, but required with higher doses to produce them. Significant palliation occurred in 60 per cent of the patients treated, but resistance developed in all cases. Results suggest that intra-arterial HN2 may be a worth-while adjunct to the conventional means of tumor therapy in selected cases.

TRANSPLANTATION OF FROZEN BROWN-PEARCE TUMOR.

MARTIN SWERDLOW* and O. SAPHIR. (Department of Pathology Michael Reese Hospital, Chicago, Ill.)

The Brown-Pearce carcinoma of rabbits, an easily transplantable tumor, can be maintained inexpensively and made readily available for a variety of research projects.

The maintenance of tumor stock in male hybrid rabbits which succumb within 4 weeks is expensive and time-consuming. Ehrlich in 1907 and others since have preserved tumors by freezing. The effect of freezing on the transplantability of Brown-Pearce carcinoma was studied.

The tumor was frozen to −72° C.; bits were thawed and transplanted usually into the testes of male hybrid rabbits. Tumors frozen 4–147 days grew and metastasized widely. The primary tumor and its metastases resulting from frozen tumor transplant were grossly and histologically indistinguishable from those resultant of fresh tumor transplant.

The successful transplantation of frozen tumor is thought by some to indicate a possible viral etiology for these tumors, since tumor cells supposedly could not survive freezing. This raises the question of the possible viral etiology of Brown-Pearce carcinoma. This is discussed, and it is concluded that a viral etiology seems unlikely, because neither cell-free filtrates nor tumors sub-

jected to chemical or physical agents which destroy the intact cell are transplantable. This is borne out by the histologic examination of frozen and thawed tumors which resist transplantation. The transplantability is due rather to the resistance of these cells to adverse influences.

ENDOCRINE REGULATION OF THE GROWTH OF THE WALKER TUMOR. PAUL TALALAY,* G. M. V. TAKANO,* and C. HUGGINS. (Ben May Laboratory for Cancer Research, University of Chicago, Chicago, Ill.)

Standardized non-necrotic tumor growth under conditions of rigidly controlled dietary intake has been obtained in 8–10 days in albino rats bearing intramuscular implants of the Walker tumor. The effects of endocrine extirpation and steroid hormones on the tumor growth rate have been studied on tube-fed animals in which adequate nutrition has maintained or increased carcass weight during the period of tumor growth. The tube feeding of large numbers of animals has been facilitated by the construction of a simple tube-feeding machine.

The effects of gonadectomy, adrenalectomy, hypophysectomy, and pregnancy on the growth rate of the Walker tumor have been studied. Adrenalectomy and hypophysectomy are both effective in retarding the rate of tumor growth under conditions of good nutrition. Cortisone acetate in large doses also causes decreased rate of tumor growth. The effects of adrenalectomy and cortisone administration are not additive. The suggestion that the adrenal secretes a growth-promoting substance other than Compound E and that hypophysectomy and cortisone acetate exert their effects via suppression of adrenal secretion will be critically evaluated.

EFFECT OF LIMITED FOOD INTAKE ON SURVIVAL OF TUMOR-BEARING MICE AND INCIDENCE OF METASTASES. ALBERT TANNENBAUM and HERBERT SILVERSTONE. (Department of Cancer Research, Medical Research Institute, Michael Reese Hospital, Chicago, Ill.)

The influence of restricted food intake on the survival of mice with spontaneous mammary carcinoma was investigated in three experiments. Strain C3H mice with small single tumors were selected and paired according to age and body weight, as well as size and location of the neoplasms. One of each pair was fed ad libitum, the other underfed. Limitation of food intake was achieved by either proportionate reduction of all dietary components or restriction of carbohydrate only. A total of 160 pairs of mice was used in the study. In comparison with the full-fed mice, those on the restricted rations lived about 15 per cent longer. Fewer developed multiple mammary tumors, and there was a lower incidence of grossly visible metastases to the lungs. The differences were of statistically significant magnitude and were present in each of the three experiments. These findings add to the biological significance of the previously reported retardation of tumor growth by caloric restriction.

In another experiment with mice bearing benzpyrene-induced sarcomas, underfeeding did not prolong the life of the animals. Although only 22 pairs were employed, it is probable that the result is valid and is a consequence of the rapid growth of this particular tumor type.

GROWTH OF HUMAN TUMORS IN THE SUBCUTANEOUS TISSUES OF X-RADIATED LABORATORY ANIMALS: THEIR PRACTICAL USE FOR EXPERIMENTAL PURPOSES. HELENE WALLACE TOOLAN. (Sloan-Kettering Institute, New York, N.Y.)

Thirty-nine or 60 per cent of 65 human epidermoid carcinomas removed surgically and immediately implanted subcutaneously in x-radiated rats of weanling age (30–40 days) became vascularized and proliferated to a size of practical use for experimental purposes. Of importance was the finding that the tumors which grew successfully did so in all the rats implanted. Growth in x-radiated hamsters was more erratic.

The tumors remained in good condition for 14-18 days before regression occurred. If removed from the rat host at approximately 14 days after implantation, they could often be transplanted successfully to new groups of x-radiated rats, to tissue culture, and to the chorio-allantoic membrane of the 8-day chick embryo.

A METHOD FOR EVALUATION OF FRACTIONATED INTRA-ARTERIAL CANCER CHEMOTHERAPY IN THE RAT. EBERHARD TRAMS,* PROSPER LOUSTALOT,* and CALVIN T. KLOPP. (Department of Surgery and The Cancer Clinic of the George Washington University Medical School, Washington, D.C.)

Fractionated regional intra-arterial cancer chemotherapy is an effective method for palliative treatment of certain malignant tumors occurring

in patients. Further evaluation of this technic has been hindered by the lack of a method which could be used in small laboratory animals in which a transplanted malignant tumor could be satisfactorily maintained. A method has now been developed which permits the study of the effects of fractionated intra-arterial cancer chemotherapy on a malignant tumor transplanted to the hind limb of the rat. This consists of the intubation of the abdominal aorta through one femoral artery with a fine indwelling polyethylene cannula. Periodic injections into this cannula may be given for as long as 15 days.

The region supplied by the injected artery has been determined by: (*a*) a knowledge of the vascular anatomy, (*b*) arteriograms obtained during injection of diatrast, and (*c*) injection of methylene blue into the cannula.

Cannuli have been inserted into a number of rats; each rat had a carcinoma transplanted into one hind limb. Daily injections of HN2 (methyl-bis(β-chloroethyl)amine hydrochloride (1.1 to 2.2 mg/kg), aureomycin hydrochloride buffered with sodium glycinate (30 to 60 mg/kg), and HN2 plus aureomycin were given. Controls received no treatment or daily injections of saline. Gross and histological changes were produced in some tumors and will be discussed. The method has been employed for a sufficiently long time to demonstrate its usefulness. However, sufficient animals have not been treated by any one dosage schedule of a given drug to permit conclusions to be drawn as to the relationship between dose and route of administration and effect.

THE PREPARATION OF 2-AMINONAPHTHALENE-5,8-C^{14} (RADIOACTIVE β-NAPHTHYLAMINE) AND THE STUDY OF ITS EXCRETION PATTERN IN DOGS, RABBITS, AND RATS.

Gray H. Twombly and Herbert Meislich.* (Department of Obstetrics and Gynecology, College of Physicians and Surgeons, Columbia University, New York, N.Y.)

The bladder carcinogen 2-aminonaphthalene (β-naphthalene) was prepared, labeled with C^{14} in the 5- and 8-position. Cinnamic acid was reduced with lithium aluminum hydride to give hydrocinnamyl alcohol, which was then converted to 1-bromo-3-phenyl-propane with phosphorus tribromide. The corresponding Grignard reagent was prepared from the bromide and then carbonated with 7 mc. of $C^{14}O_2$ to form 4-phenylbutyric acid tagged with C^{14} in the carboxyl group. The acid was ring closed to tetralone-1 with stannic chloride and reduced catalytically to the symmetrical 5,6,7,8-tetrahydronaphthalene-5-C^{14}. The hydrocarbon was carboxylated with oxalylchloride with aluminum chloride as the catalyst. Carboxylation can occur at both the 2- and 3-position, and, therefore, the compound obtained was 5,6,7,8-tetrahydronaphthalene-2-carboxylic acid-5,8-C^{14}. The latter compound was dehydrogenated with sulfur in a bomb-encased sealed tube yielding 2-naphthoic acid-5,8-C^{14}. Conversion to the 2-aminonaphthalene-5,8-C^{14} was affected via acid chloride, acid azide, and isocyanate. Sublimation of the crude base gave a pure specimen of tagged β-naphthylamine, m.p. 110.6°–111.6°C.

The radioactive amine was administered to a dog, a rabbit, and a rat. Comparative excretion patterns of the urine of a dog, a rabbit, and rats will be presented.

IN VIVO CONVERSION OF C^{14} ACETATE TO LABELED STEROIDS.

F. Ungar* and R. I. Dorfman. (Worcester Foundation for Experimental Biology, Shrewsbury, Mass.)

Patients suffering from adrenal cortical carcinoma show large increases in urinary 17-ketosteroids. Dehydroisoandrosterone is one of the major urinary metabolites. We have administered C^{14}-labeled acetate to a human female subject who has subsequently died of adrenal cortical carcinoma. The urinary steroids were isolated, and the C^{14} content was determined.

Urine specimens were extracted in the usual manner and the labeled steroids separated and purified by silica gel chromatography and crystallization. Labeled steroids isolated include dehydroisoandrosterone, etiocholanolone, androsterone, and Δ^5-androstenediol-3(β),17(β).

The steroids isolated following the administration of 0.8 mc. C^{14} acetate had an activity in the range of 2,000 counts/mg/minute. The total neutral fraction of the urine accounted for 1–2 per cent of the total administered C^{14}. Similar data are presented following the administration of varying amounts of C^{14}-acetate. The crystalline steroids were plated out on aluminum planchets and the activity determined on a Q-gas counter.

The conversion of acetate to steroids will be discussed.

CYTOLOGICAL STUDIES ON PATIENTS WITH CARCINOMA OF THE CERVIX TREATED WITH Co^{60}.

E. von Haam, C. H. Hendricks,* and T. L. Morton.* (Departments of Pathology, Gynecology and Obstetrics, and Radiology, Ohio State University, Columbus, Ohio.)

In a series of 67 patients who were treated for the established diagnosis of carcinoma of the cervix with Co^{60}, vaginal smears were obtained at frequent intervals during the treatment and up to 1 year following it. Cytological studies of the smears revealed three types of changes: those observed in malignant cells, those observed in normal epithelial cells, and those observed as the result of a constitutional reaction of the patient. Serial observations permitted the distinction between early or acute changes and delayed reactions. The cytological response to radioactive Cobalt was contrasted to those observed after roentgen treatment and radium therapy. A comparison with the clinical course of the disease following treatment with Co^{60} revealed interesting points of correlation which, with this type of therapy, seem to possess some definite prognostic value.

UNSTAINED CELL COUNTS AS A METHOD OF EVALUATING CANCEROCIDAL AGENTS. Richard O. Vycital,* Robert Schrek, and T. Howard Clarke. (Surgical Service and Tumor Research Laboratory, Research Service, Veterans Administration Hospital, Hines, Ill.)

Cellular suspensions of the transplantable Walker rat tumor 256 were incubated at 37° C. for 24 hours with moccasin venom, nitrogen mustard, adrenal cortex extract, and radioactive Au^{198}. The number of viable cells in the treated and untreated suspensions were determined by the method of unstained cell counts. The incubated mixtures were also inoculated into rats to determine tumor growth *in vivo*.

Both *in vitro* and *in vivo* studies showed that the tumor cells were killed by moccasin venom (1:10,000) and nitrogen mustard (1:30,000). In contrast, the tumor cells were resistant to the adrenal cortex extract (1:8,000). With these reagents the *in vitro* and *in vivo* experiments were in good agreement. Au^{198} (142 μc.) was not lethal to the tumor cells, according to the method of unstained cell counts, but inhibited the growth of the tumor cells when inoculated into rats. It seemed that Au^{198} was antimitotic but not cytocidal.

The unstained cell counts appeared to be a simple and rapid method for screening the cytocidal action of reagents to tumor cells.

THE ACTION OF SPLEEN EXTRACT INTRAVENOUSLY ADMINISTERED IN MALIGNANCY. George F. Watson* (introduced by Paul N. Harris). (Kitchener Clinic, Kitchener, Ontario, Canada.)

Aqueous extract of calves' spleen injected into mice bearing the Balogh sarcoma caused the regression of the tumor in a high percentage of cases (Watson, unpublished results). Later, in a collaborative study in another institution, similar results were obtained with Sarcoma 37 (Watson, Diller, and Ludwick, 1947, and Diller and Watson, 1949).

Beginning in 1929, the extract was administered subcutaneously with encouraging results to humans with advanced carcinoma. Since 1934 it has been given intravenously to patients having various types of early and moderately advanced malignancy, with the result that patients have resumed a healthy appearance, showed remarkable gains in weight, tumors have diminished in size or disappeared and in most cases there has been no recurrence even after a considerable lapse of time. In patients with advanced cancer, the results have been less marked, but the patients have shown improvement. Brief summaries of the following cases which are alive and well at this time and which were positively diagnosed by biopsy will be given: two cases of mammary carcinoma, one case of carcinoma of the uterus, one case of carcinoma of the tonsil, three cases of skin cancer, one Bowen's disease, one case of cancer of buccal mucosa, two cases of fibrosarcoma, one rectal carcinoma, and one carcinoma of the stomach with metastases diagnosed by surgical specimen.

STUDIES ON FATTY ACID OXIDATION IN TRANSPLANTED NEOPLASIA. S. Weinhouse, Arthur Allen,* and Ruth H. Millington.* (Lankenau Hospital Research Institute and The Institute for Cancer Research, Philadelphia, Pa.)

The oxidation of C^{14}-carboxyl-labeled acetate, butyrate, octanoate, and palmitate *in vitro* by slices of transplanted tumors of the mouse were compared to corresponding oxidations by liver slices from normal and tumor-bearing mice. Complete oxidation to CO_2, conversion of fatty acid carbon to acetoacetate, and the distribution of the labeled fatty acid carbon between the carbonyl and carboxyl carbons of acetoacetate were similar in both types of liver slices. In contrast to the results of others with homogenized tissue, there appeared to be no impairment in fatty acid oxidation by the intact liver cell of the tumor-bearing animal. The oxidation of acetate and butyrate, at comparable concentrations, was found to be considerably lower in tumor than in liver slices, though higher in hepatoma than in other tumors. With octanoate a marked effect of concentration

was noted. At 0.01 M, oxidation in the tumors was very low; by decreasing the concentration of the substrate to 0.005 M or lower, oxidation was restored to levels observed in liver slices. Palmitic acid was oxidized equally well by normal and neoplastic tissue slices. A marked difference between liver and hepatoma was noted in ketone body formation. In contrast to the normal liver cell, the hepatoma does not give a net production of acetoacetate *in vitro;* however, the formation of acetoacetate in very small amounts in hepatoma and in other tumors was observed by isotopic carrier technics.

THE METABOLISM OF 2-ACETYLAMINOFLUORENE IN DOGS. ELIZABETH K. WEISBURGER,* JOHN H. WEISBURGER, and HAROLD P. MORRIS. (National Cancer Institute, Bethesda 14, Md.)

Bielschowsky (Biochem. J., **39:** 287–89, 1945) isolated 2-acetylamino-7-hydroxyfluorene from the urine of rats fed 2-acetylaminofluorene (2-AAF) in an amount corresponding to about 5–8 per cent of the administered 2-AAF. In the present investigation the metabolism of 2-AAF was studied in dogs receiving the carcinogen at a level of 0.125 per cent of the diet. 2-Acetylamino-7-hydroxyfluorene, at a level of approximately 1 per cent of the ingested 2-AAF, was isolated from the ether extract of the dog urine by application of Bielschowsky's method. A synthesis of 2-acetylamino-7-hydroxyfluorene was devised which made possible the more convenient preparation of this metabolite.

2-Aminofluorene, present in the aqueous acid wash of the dog urine ether extract, was separated and purified as the acetyl derivative. This metabolite also accounted for only a small percentage of the 2-AAF fed. The presence of 2-aminofluorene showed that the dog is able to deacetylate 2-AAF.

STUDIES ON THE METABOLISM OF 2-ACETYLAMINOFLUORENE-9-C^{14}. JOHN H. WEISBURGER, ELIZABETH K. WEISBURGER,* HAROLD P. MORRIS, and HERBERT A. SOBER.* (National Cancer Institute, Bethesda, Md.)

The pathway of the radioactivity in rats intubated with labeled 2-acetylaminofluorene (AAF) in propylene glycol solution was studied in biliary-fistula rats and in pylorus-ligated animals. Approximately 40 per cent of the ingested activity was recovered in the bile in 76 hours. The urine of biliary-fistula rats accounted for about one-half as much activity as was previously found in the urine of normal rats; the feces of biliary-fistula rats were essentially free of radioactivity. These observations suggest that (*a*) following oral administration complete absorption of AAF occurs, (*b*) metabolites are excreted in the bile, (*c*) these metabolites are partially reabsorbed from the intestinal tract, and (*d*) metabolites in the feces normally originate from the bile.

After 24 hours 6 and 7 per cent, respectively, of the ingested activity was contained in the bile and the urine of a pylorus-ligated biliary-fistula rat which indicated that small amounts of the carcinogen in propylene glycol solution are absorbed through the stomach wall.

At least four metabolites are excreted in the bile and urine, as shown by radioautographs of paper chromatograms of the urine and bile from rats fed labeled AAF. One of the urinary metabolites has been identified on the chromatograms and by isotope dilution methods as being 2-acetylamino-7-hydroxyfluorene.

SOME EFFECTS OF CONCENTRATED AQUEOUS EXTRACTS OF MALIGNANT TUMORS ON CANCER PATIENTS. EMIL WEISS. (Department of Pathology, Peoples Hospital, Chicago 16, Ill.)

The extracts were prepared from a mixture of ground tumors which showed a high degree of malignancy grossly, microscopically, and a strong color reduction of dyes, described previously by us as characteristic for all malignant tumors. As a preservative, a solution of penicillin containing 200,000 units/10 cc of water was used. To 25 cc. of this solution 1–2 gm. of the ground tumor mixture was added and vigorously shaken for 10–15 minutes. The tissue extract was then filtered through several layers of linen. The extract was tested for sterility and on animals for toxicity. Every patient was further tested intracutaneously for sensitiveness to penicillin. Two cc. of the extract was administered intramuscularly once a week for 6 consecutive weeks. Five hours after the injection of the extract, every patient reacted with a chill lasting $\frac{1}{2}$–2 hours, followed by a brief rise of temperature. The patients, after a few injections, showed a marked increase of appetite, felt stronger, and increased slightly in weight. A number of patients showed remarkable changes in the accessible regional lymph nodes. After a few injections, the lymph nodes would diminish in size and become much harder. In some instances, the shrinkage was preceded by necrosis and drainage of the liquefied material. Smears were prepared from the discharges and stained with Wright's stain. The

eosinophilic granulocytes showed a definite increase. The individual epithelial cells differed considerably in size, shape, and intensity of staining.

DIPHOSPHOPYRIDINE NUCLEOTIDE REQUIREMENTS FOR OXIDATIONS BY MITOCHONDRIA OF NORMAL AND NEOPLASTIC TISSUES.

C. E. WENNER* and S. WEINHOUSE. (Lankenau Hospital Research Institute and Institute for Cancer Research, Philadelphia, Pa.)

Following our finding of a dependence on added diphosphopyridine nucleotide for oxidation of pyruvate by mitochondria of various transplanted neoplasia (Proc. Soc. Exper. Biol. & Med. [in press]), a comparison was made between tumor mitochondria and those of several normal tissues of the mouse and rat in their requirements of added DPN for oxidation of a series of Krebs cycle intermediates. DPN addition was required for oxidation of pyruvate, fumarate, malate, citrate, and α-ketoglutarate by the neoplastic mitochondria but not for oxidation of succinate. Aside from a few instances, addition of the nucleotide was not required for the initial activity of liver and kidney mitochondria; however, oxygen consumption was maintained for longer periods in its presence. Mitochondria of rat brain were similar to those of neoplastic tissues in that oxygen consumption was considerably enhanced by the addition of DPN.

In both normal and neoplastic mitochondria, DPN largely or completely replaced the ATP requirement for oxidation of the substrates under study, but oxidation rates were somewhat higher and were maintained longer when both were added. Under optimal conditions, the oxidative activities of mitochondria of neoplastic tissues on a per milligram nitrogen basis were of the same magnitude as those of the three normal tissues studied; they fell generally between those of liver and brain but were lower than those of kidney, whose mitochondria displayed the highest oxidative activity. These results suggest (*a*) that one of the possible functions of ATP in activating oxidations in cell-free systems may be to maintain the DPN level and (*b*) mitochondria of neoplastic cells have essentially the same makeup of oxidative enzymes as their normal counterparts.

THE INFLUENCE OF ROENTGEN RADIATION AND CORTISONE UPON THE TRANSPLANTABILITY OF MOUSE LEUKEMIC CELLS LINE-I_B.

ALVAR A. WERDER,* JEROME T. SYVERTON, and JACK FRIEDMAN.* (Department of Bacteriology and Immunology and the Department of Radiology, University of Minnesota, and Mt. Sinai Hospital, Minneapolis, Minn.)

Line-I_B lymphocytic leukemia has been maintained for many years by MacDowell and his associates as an inoculable line of leukemia in the highly inbred strain of mouse designated as C58. The failure of attempts in this laboratory to establish this particular line of leukemia in the highly inbred strains of mice designated as Balb, Strong A, and StoLi is in agreement with MacDowell's experience. Moreover, a heterozygous strain of Swiss albino mouse proved insusceptible. Contrariwise, Strong A, Balb, and the CFW-Swiss strains, upon treatment by whole body irradiation and cortisone acetate (Cortone, Merck), were made susceptible. Mice of these strains each in groups of twenty were irradiated, 300 r, and within 24 hours inoculated with Line-I_B leukemic cells. Death with unmistakable lesions of leukemia common mostly between the seventh and tenth days resulted in all mice of strains Strong A, Balb, and Swiss. The control mice survived irradiation or leukemic cells in the same dose. The effects differed somewhat when cortisone was employed. Mice of the same strains which had received cortisone, 0.05 mg., daily for seven injections were tested for susceptibility to Line-I_B leukemic cells. The proportion of mice (*a*) of Balb strain that died was 15/15, (*b*) CFW-Swiss, 5/18, in contrast to (*c*) survival for all twelve Strong A mice. The control mice survived leukemic cells or cortisone. Finally, most mice of resistant Strong A, Balb, and Swiss albino strains previously immunized by the injection of Line-I_B leukemic cells upon treatment with x-ray or cortisone were found insusceptible to Line-I_B leukemia. These latter findings suggest that specific acquired immunity is more effective as a protective mechanism than natural innate resistance or genetic constitution.

AN OBSERVED CORRELATION BETWEEN PSYCHOLOGICAL FACTORS AND GROWTH RATE OF CANCER IN MAN.

PHILIP M. WEST,* EUGENE M. BLUMBERG,* and FRANK W. ELLIS.* (Department of Biophysics, School of Medicine, University of California at Los Angeles, and Departments of Psychology and Investigative Medicine, Long Beach Veterans Administration Hospital, Long Beach, Calif.)

In an attempt to determine what part, if any, stresses of emotional origin might play in the growth behavior of human cancer, extensive psychological testing was carried out on a number of

patients. The experimental design consisted of setting up two contrasting groups of cases with unusually rapid or unusually slow progression of their disease in relation to the average for the particular neoplasm. These groups were then studied for differentiating personality factors. While many standard psychological tests were used, this report concerns only the data obtained with the Minnesota Multiphasic Personality Inventory, since its simplicity and objectivity eliminate the factor of personal interpretation by the examiner, and the results may be accurately reproduced by others.

By this method it was found that the two medical groups of cancer patients could be distinguished with an accuracy of 88 per cent. Statistical analysis revealed that the correlation between certain readily measured psychological factors and neoplastic activity was significant beyond the 1 per cent level. The possible implications of these findings and the use of this approach in predicting responsiveness to cancer therapy will be discussed.

THE EXCRETION RATE OF UROPEPSIN BY PATIENTS WITH CARCINOMA, LYMPHOBLASTOMA, AND LEUKEMIA.

PHILIP M. WEST and FRANK W. ELLIS. (School of Medicine, University of California at Los Angeles, and Long Beach Veterans Administration Hospital, Long Beach, Calif.)

There is considerable evidence to indicate that the excretion rate of uropepsin is under hormonal control, mediated via the adrenal cortex. Simplified methods for measuring the urinary output of this enzyme make it technically feasible to obtain daily information on adrenal activity in considerable numbers of patients. Uropepsin excretion studies were done on 80 individuals with neoplastic disease, both before and during radiation and chemotherapy.

Very high uropepsin outputs with great daily fluctuations were found in the acute leukemias and in rapidly progressing cases of carcinoma and Hodgkin's disease which were generally resistant to therapy. Lymphocytic sarcomas and leukemias formed a striking exception, the same excretion pattern being associated with low-grade neoplastic activity or actual temporary spontaneous regression. In all groups of neoplasms, consistently low uropepsin values were found in those cases whose disease could be most readily controlled by palliative means for long periods. The relationship between ACTH administration, uropepsin excretion rate, and therapeutic effect will be presented.

EFFECT OF X-RADIATION ON LYMPHOID TISSUE NUCLEIC ACIDS IN C57 BLACK MICE.

PATRICIA P. WEYMOUTH* and HENRY S. KAPLAN. (Department of Radiology, Stanford University School of Medicine, San Francisco, Calif.)

Whole body x-radiation was administered to C57bl mice at a dose level known to produce thymic lymphomas in 80–90 per cent of mice of this strain. These mice, with untreated litter-mate controls, were sacrificed at 4, 30, 60, and 90 days after the last treatment. Nucleic acids were separated from thymus and axillary lymph nodes according to Schmidt and Thannhauser. The phosphorus content of the desoxyribonucleic acid (DNAP) and combined ribonucleic acid and phosphoprotein (residual, RP) fractions was determined by the method of Berenblum and Chain.

In untreated controls aged 35, 62, 90, 120, and 150 days, RP concentration did not change appreciably with age and was the same in thymus and lymph nodes. The concentration of DNAP in both tissues increased to a maximum at about 90 days, decreasing thereafter, and was higher in the thymus than in the lymph nodes by a factor of 1.3–2.0.

In irradiated mice, thymic DNAP concentration was decreased about 40 per cent 4 days after treatment and remained significantly reduced for as long as 90 days. Lymph node DNAP concentration was decreased at 4 and 30 days after irradiation but had returned to the control level at 90 days.

There was no change in RP concentration after x-radiation in either thymus or lymph nodes.

NITROGEN EXCRETION FOLLOWING WHOLE-BODY X-RADIATION.

JULIUS WHITE and BERNARD E. BURR.* (National Cancer Institute, Bethesda, Md.)

It has been reported (Prosser *et al.*, Radiology, **49**: 299, 1947) that following whole-body x-radiation of dogs, the urinary excretion of nitrogen remained at fairly normal levels, even though nitrogen intake was markedly reduced.

This report deals with preliminary results of our study of the effect of whole-body irradiation on nitrogen metabolism in rats.

Osborne-Mendel rats weighing 180–210 gm. were allowed to consume ad libitum a diet of the following percentage composition: casein, 6; starch, 47.5; Crisco, 24; dried brewers yeast, 2; sucrose, 15; inorganic salts, 4; cod liver oil, 1; and cystine, 0.5. When the daily nitrogen excretion was

constant, each rat received a whole body x-radiation of 450 r.

The first 24-hour sample of urine showed a twofold increase in nitrogen excretion which was reflected chiefly in the urea fraction. The nitrogen remained elevated for 3–4 days following irradiation and then returned to normal. Associated with this increase in nitrogen excretion was a decrease in food consumption but not of the magnitude to account for the high nitrogen excretion.

CONCERNING THE *IN VITRO* METABOLISM OF LABELED CARCINOGENIC HYDROCARBONS. WALTER G. WIEST* and CHARLES HEIDELBERGER. (McArdle Memorial Laboratory, University of Wisconsin, Madison, Wis.)

In order that studies of the metabolism of carcinogenic hydrocarbons might be more easily controlled and contaminants decreased, it was thought desirable to develop an *in vitro* system. In all the following experiments the reaction mixtures after incubation with dibenzanthracene-9,10-C^{14} were analyzed for metabolites by known liquid extraction procedures, filter-paper chromatography, and carrier crystallizations to determine unchanged dibenzanthracene. Slice experiments were run in Robinson's glucose medium; homogenates were fortified (cf. Mueller and Miller, J. Biol. Chem., **180:** 1125, [1949], with pyruvate and fumarate in addition), and adequate oxygen uptake was maintained for 3 hours. The following experiments were carried out:

1. Skin slices from suckling and weaned mice and slices of liver and submaxillary glands of young adult mice were incubated with colloidal dibenzanthracene-9,10-C^{14}. Normal oxygen uptake of liver was not inhibited by dibenzanthracene.

2. Slices of livers of mice injected intravenously with dibenzanthracene-9,10-C^{14} 15 minutes prior to death were incubated.

3. Liver slices were incubated with dibenzanthracene-9,10-C^{14} solubilized by "Triton X-100," which inhibited the oxygen uptake.

4. Slices from a mouse injected with nonradioactive dibenzanthracene 13 hours before death were incubated with labeled colloidal and "Triton"-treated dibenzanthracene.

5. Colloidal dibenzanthracene-9,10-C^{14} was incubated with homogenates of liver and submaxillary gland.

While it is possible that up to 1 per cent of the hydrocarbon was metabolized in some of these experiments, the amount was too slight to be considered significant.

The experiment of Weigert *et al.* (Nature, **158:** 417, 1946), who obtained metabolism of benzpyrene in mouse skin floated in Ringer-Locke solution, was confirmed with benzpyrene-5-C^{14}; however no metabolism of dibenzanthracene-9,10-C^{14} was obtained under the same conditions.

STUDIES ON THE INTERACTION OF LABELED CARCINOGENIC HYDROCARBONS WITH TISSUE COMPONENTS. WALTER G. WIEST* and CHARLES HEIDELBERGER. (McArdle Memorial Laboratory, University of Wisconsin, Madison, Wis.)

Skins from the backs of mice were frozen in liquid air. Most of the dermis was scraped off, leaving intact the epidermis and that part of the dermis containing sebaceous glands and hair follicles. This tissue was soaked in dilute acetic acid and homogenized. The homogenate was separated by centrifugation into particulate and supernatant fractions.

When carcinogenic hydrocarbons were injected into submaxillary glands of mice, a high incidence of tumors was produced within a short time. Submaxillary glands were homogenized and separated by differential centrifugation into nuclear, large granule, small granule, and supernatant fractions. These fractions from skin and submaxillary gland were analyzed separately for nucleic acids. Radioactivity was determined before and after the protein was precipitated with TCA and washed.

Following administration of dibenzanthracene-9,10-C^{14}, more radioactivity was found in the particulate fraction of skin than in the supernate. None of the radioactivity associated with the particulate skin protein 2 weeks after application was removed by organic solvents or boiling 5 per cent TCA. Radioactivity was found in all fractions of submaxillary gland 2 weeks after injection of dibenzanthracene-9,10-C^{14}. The largest amount occurred in the small granules. Most of the C^{14} was extracted from the precipitated protein by organic solvents; however, a significant amount, highest in the small granule fraction, was not removed by organic solvents, boiling 5 per cent TCA, or 0.1 N NH_4OH. The nature and extent of this binding is under further investigation in skin, submaxillary gland, and tumors, using both dibenzanthracene-9,10-C^{14} and benzpyrene-5-C^{14}.

OCCURRENCE OF LYMPHOSARCOMA IN RATS FED WITH CERTAIN DYE SUBSTANCES. R. WILLHEIM and A. C. IVY.*

(University of Illinois, Department of Clinical Science, Chicago, Ill.)

During an investigation concerning absorption and deposition of dye substances in the mucosa of the gastro-intestinal tract of rats, the following observation was made: Some of the dyes occasionally induced the development of extensive multiple lymphatic tumors originating from retroperitoneal and mesenteric lymph glands. The histologic picture at post mortem was that of lymphosarcoma. The dyes were fed as a 4 per cent admixture to ground "Purina Fox Chow." The tumors developed after 6–20 months of feeding. The effective dyes were: the natural dye hematoxylin and the registered dyes: Amaranth (F. D. & C. Red #2), Guinea Green B (F. D. & C. Green #1), and Ponceau SX (F. D. & C. Red #4). Among the 30 animals which lived up to the minimum tumor age of 6 months' feeding time and belonged to the respective experimental groups, four tumors occurred so far. Among a control group of 50 animals, no tumor occurred. The authors are inclined tentatively to ascribe the development of these tumors to a chronic strain on the lymphatic apparatus, especially in the abdomen, or to a damage of the reticuloendothelial cells of the area.

ACUTE LIVER CHANGES PRODUCED BY 3′-METHYL-4-DIMETHYLAMINOAZOBENZENE IN TWEEN 80. G. Z. Williams. (Department of Oncology, Medical College of Virginia, Richmond, Va.)

White rats were injected intraperitoneally with 3′-methyl-4-dimethylaminoazobenzene, either as a single dose of 50 mg. or twice weekly for 4–6 weeks in doses of 12.5 mg. each. At intervals after injection, a group of animals was sacrificed, and the peritoneal fluid, peritoneal tissue, liver, adrenal, spleen, and kidneys were examined for pathologic changes. The changes found in the livers indicate acute azo dye poisoning. The histological changes and alteration of glycogen, protein, and fat content of the liver cells will be described. A series of control animals receiving equivalent amounts of Tween 80 only were included, and no significant changes were found. The LD_{50} is approximately 50 mg/100 gm for rats.

INTRAPERITONEAL ADMINISTRATION OF AZO DYES IN TWEEN 80. G. Z. Williams (assisted by R. D. Yarborough). (Department of Oncology, Medical College of Virginia, Richmond, Va.)

A number of spreading agents, detergents, and soaps were tested in an attempt to suspend dimethylaminoazobenzene and its 3′-methyl derivative in order to provide a suitable vehicle for intraperitoneal injection. Tween 80 (Atlas Powder Company) was tolerated well when given by intraperitoneal injection to rats. Doses as high as 1 ml/day for 5–7 days and single doses of 4 ml. were well tolerated. Preliminary experiments indicate an MLD of approximately 10 ml. which may be due to mechanical effects. 3′-Methyl-4-dimethylaminoazobenzene suspends easily in Tween 80 when heated, and after cooling as much as 5 per cent can be maintained in satisfactory suspension for considerable periods of time. Suspensions of 2.5 per cent are stable for several days. It was found that intraperitoneal injection of the azo dyes in Tween 80 provided a suitable method for administration of relatively large doses of these agents with uniform distribution in the peritoneal cavity and marked absorption by the omental and retroperitoneal fat.

EFFECT OF 3′-METHYL-4-DIMETHYLAMINOAZOBENZENE AND RADIOGOLD COLLOID ON RAT LIVER. G. Z. Williams (assisted by M. L. Haigler and R. D. Yarborough). (Department of Oncology, Medical College of Virginia, Richmond, Va.)

Rats were injected with radioactive gold colloid by splenic or intracardiac route 7–10 days before administration of 3′-methyl-4-dimethylaminoazobenzene. The animals were divided into two groups: one group received the azo dye in Tween 80 by intraperitoneal injection 2 times weekly for 4–6 weeks and were fed a basal synthetic diet without azo dye; the second group was fed the basal synthetic diet which included 0.04 per cent of the azo dye. Histological and histochemical evidence of damage to the liver cells will be reported and a type of small cell regeneration of liver described. The results include a higher incidence of hepatomas in the animals fed azo dye after administration of radiogold colloid. The findings are interpreted in relation to the apparent co-carcinogenic activity of isotope beta radiation.

EFFECT OF RADIOGOLD COLLOID ON THE GROWTH OF HS-1 ASCITES TUMOR IN MICE. G. Z. Williams and J. T. Williams* (assisted by M. L. Haigler). (Department of Oncology, Medical College of Virginia, Richmond, Va.)

HS-1 ascites tumor was transferred in free cell form from the peritoneal cavities of donor mice into three groups of animals. In Group 1, the mice had previously been injected with intraperitoneal

radiogold colloid, 0.1 mc/week for 5 weeks, the last dose 1 week before transfer of tumor cells; Group 2 received 0.2 mc. of radiogold colloid with the tumor cell transfer; and in the third group the animals were injected intraperitoneally with 0.2 mc. of the radioactive sol 48 hours after the free cell tumor had been transferred. Growth curves will be shown indicating the effect of the radiogold in reducing growth of the free tumor cells and in controlling implantation of the tumor cells on the peritoneal surfaces. Charts will be shown to depict the relation of effective radiogold doses and the number of tumor cells transferred.

METHIONINE-S^{35} UPTAKE BY HS-1 ASCITES TUMOR CELLS. G. Z. Williams, J. T. Williams,* and M. L. Haigler.* (Department of Oncology, Medical College of Virginia, Richmond, Va.)

Tracer doses of methionine-S^{35} were included in an amino acid mixture which was injected into the peritoneal space of albino mice previously inoculated with HS-1 ascites tumor. At periodic intervals the animals were sacrificed and the peritoneal content of fluid and free cells removed. The washed tumor cells were counted for total activity of methionine-S^{35} taken up. An aliquot was homogenized, and cytoplasmic fractions and nuclei were separated by centrifugation. These fractions were counted with a gas flow counter to determine the S^{35} activity of each portion. These experiments were repeated with daily injections of methionine-S^{35} in order to compare the rate of uptake by the tumor cells when the radioactive amino acid supply was maintained on a daily level with that found after single dose administration. The results will be depicted in graphic form.

THE EFFECT OF A SERIES OF ETHYLENE AMINES AGAINST EXPERIMENTAL CANCER. J. H. Williams,* Doris McKenzie,* Sybella Halliday,* Gordon R. Personeus,* Marion L. Stevens,* Sarah J. Sparks,* Samuel G. Smith,* William P. Troy,* Hester S. Schurr,* Harold R. Gleason,* Eleanor R. James,* Lawrence Moser,* Patricia Lydick,* M. Jane Landes,* Virginia Eve,* Patricia Stick,* and Norma Vincent* (introduced by Sidney Farber). (Research Department, Lederle Laboratories Division, American Cyanamid Company, Pearl River, N.Y.)

In studying the carcinolytic effect of chemicals against transplanted cancer of mice and rats, phosphoric acid triethylene imide and phosphoric acid diethylene imide were reported to be as active as and less toxic than triethylene melamine. When this study was extended, the relative instability of these chemicals became evident. Accordingly, it was of interest to study the chemotherapeutic possibilities of others' having a close structural relationship. Therefore, we have studied a series provided by the research laboratories of Calco Chemical Division, American Cyanamid Company.

Each member of this series showed a marked degree of carcinolytic effect against transplanted sarcomas and carcinomas with phosphoric acid diethylene imide, phosphoric acid triethylene imide, N-pentamethylene-N′-N″-diethylene-phosphoramide and N,N′,N″-tris(methyl-ethylene)-phosphoramide, N-(3-oxapentamethylene)-N′-N″-diethylenephosphoramide and octyl N,N′-diethylenediamidophosphate showing, in addition, a most marked effect against leukemia. Therefore, this series was studied from the point of view of stability and optimum treatment doses; and three additional products which seemed to possess reasonable stability, namely, N-pentamethylene-N′-N″-diethylenephosphoramide, N-(acrylamidomethyl)-3-ethylene-iminopropionamide, and N,N′,N″-tris(2-methylethylene)phosphoramide were selected as worthy of initial clinical studies, following additional pharmacological study.

PRODUCTION AND CHARACTERISTICS OF A NEW ASCITES TUMOR IN MICE. J. T. Williams* and G. Z. Williams. (Department of Oncology, Medical College of Virginia, Richmond, Va.)

A transplantable spontaneous carcinoma of the breast was found in an albino mouse of the colony in our laboratory. This tumor has been maintained by serial transplantation for the past 4 years, and it is characterized histologically by highly anaplastic cells resembling sarcoma and grows readily in all strains of mice tested. This tumor is designated HS-1. Homogenates of freshly removed young tumor (without necrosis) were suspended in Ringer's solution, and doses of approximately five- to ten-million cells were injected into the peritoneal cavities of albino mice. The tumor grew readily in "free cell" form in many of the injected mice. Fluid containing tumor cells was removed and serially transferred in albino and C3H mice. More than 65 serial transfers of the free tumor cells in peritoneal fluid have been made and the growth characteristics studied. These will be described and illustrated with phase photomicrographs to depict the nature of this highly malignant ascitic tumor.

NUCLEAR INCLUSIONS IN SPONTANEOUS AND INDUCED HEPATOMAS AND IN SERIAL TRANSPLANTS OF HEPATOMAS IN MICE.

J. Walter Wilson. (Department of Biology, Brown University, Providence 12, R.I.)

Nuclear inclusions have been frequently reported in the liver cells of apparently normal mice. They are eosinophilic spheres, with a basophilic shell, sometimes so large as to fill the nucleus, displacing its chromatic elements to a narrow space under the nuclear membrane. Cowdry placed them, as described by Findlay, among his Class B virus inclusions.

We have formed them in large numbers in mice of our own BUB strain, fed diets containing *o*-aminoazotoluol, or 3′-methyl-4-dimethylaminoazobenzene, not only in cells of the liver but also in the tumors. In some tumors they are exceedingly abundant. We have also found them in spontaneous hepatomas of C3H mice and in serial transplants of them, and in hepatomas produced by repeated doses of CCl_4.

Since no pathological condition has been associated with these inclusions, it has not been proved that they are virus inclusions. However, even if so, it is doubtful whether they play any significant role in carcinogenesis, for they are not found in some tumors, and they are found occasionally in livers of normal animals and in increased abundance in livers of animals subjected to long treatment of drugs not known to be carcinogens, e.g., Coramine and thiouracil. It is suggested that, if they are due to a virus, their occasional abundance in hepatomas and transplanted hepatomas may be due to some peculiar physiological condition of the particular tissues in which they occur that is favorable to the growth of the virus.

I^{131} UPTAKE BY TRANSPLANTABLE THYROID TUMORS IN THE C3H MOUSE.

S. H. Wollman,* R. O. Scow,* B. Wagner,* and H. P. Morris. (National Cancer Institute and National Institute of Arthritis and Metabolic Diseases, Bethesda, Md.)

Larger tumors of the more active of four lines studied had total I^{131} uptakes many times that of the thyroid gland. When uptake by either thyroid gland or tumor was high, there was an accompanying decrease in tumor or thyroid uptake, respectively. Uptake by tumor was proportional to mass in cases of tumor uptakes of less than 30 per cent. The ratio of uptake per milligram of tumor to that of thyroid in more active tumor lines was a constant, independent of time, for intervals studied (2 to 49 hours). Tumors were always less active than thyroids on this basis and had activities of $\frac{1}{10}$, $\frac{1}{200}$, or less than $\frac{1}{200}$, that of the thyroid, thus providing a method of classification of these tumors.

These observations are consistent with a theory developed by Keating and by Pochin (for competing thyroid gland and kidneys in man), if generalized to include tumors competing for circulating I^{131}, and provided that the clearance by the tumor is proportional to tumor mass with a proportionality constant characteristic of the tumor line.

The results suggest the need for reanalyzing clinical data in cases where it has been claimed that removal of functional tissue has induced a large increase in metastatic tumor I^{131} uptake by virtue of increased production of endogenous TSH. It is desirable to determine how much of the observed increase might be due to removal of a competitor or to increase in tumor mass.

ETIOLOGIC FACTORS OF BRONCHIOGENIC CARCINOMA IN PHYSICIANS.

Ernest L. Wynder and Harold Jeghers.* (Department of Medicine, Memorial Center for Cancer and Allied Diseases, New York, N.Y.

In line with a previous investigation of etiologic factors of primary cancer of the lung in the general population, a special survey was carried out among a population group considered to be free from specific exposures to occupational fumes and irritants. Physicians were believed to represent such a group.

Etiologic factors studied included those of city dust, possible exposures to exogenous irritants in hobbies, and consumption of tobacco. Of these factors only tobacco showed significant differences between a group of 60 physicians with cancer of the lung and a group of 150 physicians with cancer outside of the respiratory tract.

There are significantly fewer non- and light smokers among physicians with cancer of the lung than among physicians with types of cancer outside the respiratory tract. Conversely, physicians with cancer of the lung include a significantly greater number of excessive and chain smokers than were found in the control group.

These data tend to confirm the conclusion from previous investigations that tobacco is a factor of importance in the production of primary cancer of the lung.

HISTOCHEMICAL STUDIES ON THE REGENERATING MOUSE LIVER. Hisako O. Yokoyama,* Kenneth K. Tsuboi, Margaret E. Wilson,* and Robert E. Stowell. (Department of Pathology and Oncology, University of Kansas Medical School, Kansas City, Kan.)

Partial hepatectomy was performed on strain A male mice, and a study was made of the regenerating liver at various times, correlating the chemical, histochemical, and quantitative cytological data. Tissues were collected at intervals of ¾, 1, 2, 3, 4, 5, 6, 7, 8, 10, 14, 21, and 28 days, 4 months and 6 months after partial hepatectomy. Several animals were used for each group, and analyses were made on individual samples. This report will consider the histochemical observations on tissues prepared by freezing-drying and other chemical fixation methods.

The changes in distribution of glycogen, nucleic acids, lipids, alkaline phosphatase, acid phosphatase, and esterase were studied. Large amounts of lipids are mobilized into the liver cells in the first few days of regeneration. Liver cells in mitotic division contain less glycogen.

Marked changes occur in the first several days after partial hepatectomy, some of these being correlated with the peak of mitotic activity which appears on the third day. Ribonucleic acid is definitely increased during the period of rapid regeneration of cells. Alkaline and acid phosphatase activity is also appreciably increased. Nondeparaffined sections of regenerating cells at certain stages show a dark perinuclear reaction for acid phosphatase with Gomori's technic. The changes in amount and distribution of various substances observed histochemically will be compared to quantitative biochemical analyses calculated on the basis of amounts per cell, as well as other standard base lines.

THE *IN VIVO* METABOLISM OF AZO DYES. Nelson F. Young and R. D. Yarborough.* (Department of Oncology, Medical College of Virginia, Richmond, Va.)

The introduction of Tween 80 solutions of azo dyes into the peritoneal cavity of animals provides an opportunity for observing the metabolism of these dyes in a convenient manner. One week after the injection of 50 mg. of dye by this means into the rat, the retroperitoneal fat still contains large amounts of the original dye and various fat-soluble metabolites. At least one of these metabolites has not been previously reported. This report is concerned with a comparison of the metabolic products of (*a*) carcinogenic and noncarcinogenic azo dyes in rats, (*b*) carcinogenic dyes in full-fed and deficient rats, and (*c*) carcinogenic dyes in rabbits, guinea pigs, and mice.

AUTHOR INDEX

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